Sentinel Laboratory Manual For Agents of Bioterrorism

Bacillus anthracis
***Brucella* spp.**
***Burkholderia* spp.**
Clostridium botulinum
Coxiella burnetii
Francisella tularensis
***Staphylococcus* Enterotoxin B**
Variola major virus
Yersinia pestis

Utah Department of Health, 10/2004

SENTINEL LABORATORY MANUAL FOR THE AGENTS OF BIOTERRORISM

TABLE OF CONTENTS

NOTIFICATION PROCEDURES FOR SENTINEL LABORATORIES
FOR POSSIBLE BIOTERRORISM INCIDENTS

When should the lab call the State and/or Local Health Department?

The State and Local Health Departments should be notified and the organism sent to the Utah Department of Health Laboratory (UDOH) if:

1. The lab presumptively isolates one of CDC's high priority BT agents (e.g. *Bacillus anthracis, Francisella tularensis, Yersinia pestis, Brucella, Clostridium botulinum, Coxiella burnetii, Burkholderia mallei, Burkholderia pseudomallei, and* Variola virus).
2. A physician requests a culture for one of these agents based on a high index of suspicion (actually suspects the disease and is not just ruling out a possibility)
3. Large numbers of identical isolates are isolated from persons with similar disease or syndromes (e.g. *Salmonella, Shigella, etc.).*
4. The lab isolates an uncommon organism that causes a disease not normally present in Utah.
5. An organism exhibiting unusual biochemical or antibiotic susceptibility patterns.

If an agent is used as a bioterrorism agent in a covert or "unannounced" event, immediate and appropriate notification will help save others in the community. If one of the high priority BT agents is suspected, specimens should be split with one set forwarded to the UDOH for rapid/advanced culturing. To ship to the reference laboratory (UDOH), pack the presumptive BT agent according to the protocol for packing and shipping infectious agents (see shipping protocol). Contact the UDOH for transport assistance and to let them know the specimen/isolate is being sent. The suspected BT specimens/isolate will be confirmed at the UDOH or referred to another higher-level laboratory. In a confirmed bioterrorism event, all handlers must be identified on a chain-of-custody form.

The UDOH will report test results to the submitting laboratory, Epidemiology, CDC and other appropriate agencies.

When should the FBI and local law enforcement also be notified?

Specifically, the FBI must be notified for one or more cases, definitively diagnosed with one or more of the following:

1. Smallpox or pulmonary anthrax
2. Uncommon agent or disease (e.g. *Burkholderia mallei* or *pseudomallei*) occurring in a person with no other explanation
3. An illness caused by a microorganism with markedly atypical features (may indicate the organism was genetically altered).
4. An illness due to aerosol, food, or water sabotage, as opposed to a usual transmission route.
5. If an environmental specimen such as a package or letter is received, they should not be opened. Call 911, and local HAZMAT teams will package these samples for analysis.

CONTACT INFORMATION

UTAH DEPARTMENT OF HEALTH
This Level A Manual and UDOH Lab Client Services Manual are available at:
www.health.utah.gov/els/microbiology

- **Emergency #: Pager: 888-EPI-UTAH**
- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-538-6246,**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**
- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: **rrolfs@utah.gov**

LOCAL LAW INFORCEMENT: Call 911

LOCAL FBI
Office: 801-579-1400
Contact: Chip Spencer

LOCAL HEALTH DEPARTMENTS

Bear River Health Department: 435-792-6500, Fax: 435-792-6600
Director: John Bailey MD: 435-792-6482, email: **johnbailey@utah.gov**

Central Utah Health Department: 435-896-5451, Fax: 435-896-4353
Director: Robert Resendes: Extension 17, pager: 801-202-7955
email: **rresendes@utah.gov**

Davis County Health Department: 801-451-3340, Fax: 801-451-3242
Director: Lewis Garrett: 801-451-3351, email: **lewisg@co.davis.ut.us**
Disease Reporting - 801-451-3003
24-Hour Pager - 801-241-8318

Salt Lake Valley Health Department: 801-534-4600, Fax: 801-534-4557
Director: Patty Pavey: 801-468-2755, pager: 801-202-7535
email: **ppavey@my2way.com**
Epidemiology: Ilene Risk: 801-534-4638
Surveillance: Mary Hill: 801-534-4528, cell 801-647-5471
email: **mhill@slco.org**

Southeastern Utah District Health Department: 435-637-3671, Fax: 435-637-1933
Director: David Cunningham: cellular: 435-637-3671, pager: 801-202-7588
email: **dcunning@utah.gov**

Southwest Utah Health Department: 435-673-3528, Fax: 435-628-6713
Director: Gary Edwards: Extension 20, pager: 801-202-7121
email: **gedwards@utah.gov**

Summit County Health Department: 435-336-3222, Fax: 435-336-3286
Health Officer: Steve Jenkins: 435-336-3223, pager: 801-202-7061,
email: **sjenks@utah.gov**

Tooele County Health Department: 435-843-2300, Fax: 435-843-2304
Director: Myron Bateman: 435-843-2305, pager: 435-833-1892,
email: **mbateman@utah.gov**

Tri-County Health Department: 435-781-5475 Fax: 435-781-5372
Director: Joseph Shaffer: 435-781-5475, cellular: 801-202-7096
email: **jshaffer@utah.gov**

Utah County Health Department: 801-851-7000, Fax: 801-343-8709
Director: Joseph Miner M.D.: 801-851-7011, pager: 801-612-7119,
email: **uchlth.joem@state.ut.us**

Wasatch County Health Department: 435-654-2700, Fax: 435-654-2705
Director: Phil Wright: Extension 3250, pager: 801-202-7098
email: **philw@utah.gov**

Weber Morgan Health Department: 801-399-8433, Fax: 801-399-8306
Director: Gary House: 801-399-8463
email: **ghouse@co.weber.ut.us**

AGENTS OF BIOTERRORISM
GENERAL ORGANISM INFORMATION

BACILLUS ANTHRACIS

Bacillus anthracis is an aerobic, spore forming, non-motile, large, Gram-positive rod. Humans can become infected with *B. anthracis* by handling products or consuming undercooked meat from infected animals. Infection may also result from inhalation of *B. anthracis* spores from contaminated animal products, such as wool, or the intentional release of spores. Human to human transmission has not been reported.

Three forms of anthrax occur in humans:

Cutaneous anthrax
Cutaneous infection is the most common form in the United States, and accounts for 95% to 99% of all cases worldwide. The organism enters through an abrasion in the skin, and infection begins as a small papule that is often pruritic. The papule enlarges and within 24-48 hours develops into an ulcer surrounded by vesicles. A **black necrotic central eschar (scab)** develops later and is characteristic. Edema is often striking and **the lesion is painless**. Untreated cutaneous anthrax is fatal about 20% of the time due to septicemia and meningitis. Deaths are rare after antimicrobial treatment. The bacilli are readily recognized in the serosanguinous discharge.

Inhalation anthrax
Infection is caused by inhalation of spore-laden dust; dust from hides, wool, and mohair constitutes the primary source of infection in industry. Inhalation anthrax is also the most likely following an intentional aerosol release of *B. anthracis*. Direct human-to-human transmission is very rare. After an incubation period of 1-6 days, presumably dependent upon the dose of inhaled organisms, the onset of inhalation anthrax is gradual and nonspecific with fever, malaise, and fatigue. After 2-3 days, severe respiratory distress develops with severe hypoxia, dyspnea, and cyanosis. Shock and death usually follow within 24-36 hours. Physical findings are nonspecific. Characteristically, the chest X-ray may reveal a widened mediastinum and pleural effusion. The mortality rate approaches 100% despite aggressive treatment.

Gastrointestinal anthrax (and oropharyngeal anthrax)
The gastrointestinal form may follow the consumption of contaminated meat from infected animals and is characterized by an acute inflammation of the intestinal tract. Presentations may vary and include gastrointestinal pain, bleeding, and rapid development of ascites. The fatality rate is 25 to 60% if not treated. The organism may be isolated from the patient's stools.

AGENTS OF BIOTERRORISM
GENERAL ORGANISM INFORMATION

BRUCELLA SPP.

Brucella is a fastidious, aerobic, small, gram-negative coccobacillus. It is typically a zoonotic infection, with four species being recognized as causing infection in humans: *Brucella abortus* (cattle), *Brucella melitensis* (goat, sheep, and camels), *Brucella suis* (pigs), and *Brucella canis* (dogs).

Brucella can cause both acute and chronic infections. The symptoms of Brucellosis are specific and systemic, with fever, sweats, headache, anorexia, back pain, and weight loss being frequent. The chronic form of the disease can mimic miliary tuberculosis with suppurative lesions in the liver, spleen, and bone. The organism is often included in the differential diagnosis of fevers of unknown origin. It has a mortality of 5% in untreated individuals.

BURKHOLDERIA MALLEI

Burkholderia mallei is a gram-negative, non-motile coccobacillus or slightly curved rod with rounded ends and appears either singly, in pairs end-to-end, or in parallel bundles. *B. mallei* is the etiologic agent of Glanders, a contagious and rare disease of equines, which is often fatal when transmitted to humans.

Burkholderia mallei causes necrosis of the tracheobronchial tree, pustular skin lesions, febrile pneumonia or sepsis, and abscesses depending upon the mode of infection. A widespread necrotizing skin eruption has been described as a terminal feature in patients with disseminated Glanders. As a bioterrorism agent, it would most likely be disseminated as an aerosol, but could also be introduced through food or water. The mortality rate is over 50% despite treatment.

BURKHOLDERIA PSEUDOMALLEI

Burkholderia pseudomallei is a gram-negative aerobic bacillus that grows well on routine culture media. It is motile and colonies vary from smooth and mucoid to dry and wrinkled. *B. pseudomallei* is the etiologic agent of Melioidiosis, a disease of humans and other mammals.

Two common clinical presentations are ‘septicemia Melioidiosis’ (also known as community acquired ‘sepsis syndrome’), and as ‘localized Melioidiosis’ (lung, parotid gland, etc.). Symptoms in patients with sepsis syndrome include high fever, rigors, confusion, stupor, jaundice, diarrhea, leucocytosis, coagulopathy, evidence of renal and hepatic impairment, and abnormal chest x-ray (nodular shallowing). These clinical symptoms are found in 60-80% of patients. Many patients die within 48 hours of hospital admission. Localized infections present with cutaneous/subcutaneous abscesses and lymphadentitis. It frequently occurs in the lung with abscesses and profound weight loss, which may be confused with tuberculosis.

AGENTS OF BIOTERRORISM
GENERAL ORGANISM INFORMATION

CLOSTRIDIUM BOTULINUM

Botulism is a neuroparalytic disease produced by the neurotoxins of *C. botulinum*, *C. argentinense* (*C. botulinum* type G), *C. butyricum* (type E) and *C. baratii* (type F). Seven distinct toxin types are produced by the organisms: A, B, C, D, E, F, and G. Types A, B and E, are most commonly associated with disease in the United States. Botulinum toxin types C and D cause disease in birds and mammals. Botulinum toxin type E is usually associated with fish products. Clostridia are obligately anaerobic, spore forming, straight to slightly curved, bacilli that usually stain Gram positive. Spores of the organisms are ubiquitous in soil, are found in the intestines of animals including man and are present in marine and aquatic sediments.

Neurological symptoms and signs dominate the clinical syndrome of botulism. Incubation periods for foodborne botulism are reported to be as short as 6 hours or as long as 10 days, but generally the time between toxin ingestion and onset of symptoms ranges from 18-36 hours. The ingestion of other bacteria or their toxins in the improperly preserved food or changes in bowel motility are likely to account for the abdominal pain, nausea, vomiting, and diarrhea that often precede or accompany the neurological symptoms of foodborne botulism. Dryness of the mouth, inability to focus to a near point, and diplopia (double vision) are usually the first complaints. If the disease is mild, no other symptoms may develop and the initial symptoms will gradually resolve. In more severe cases, the initial symptoms may be followed by voice impairment (dysphonia), difficulty swallowing (dysphagia), and peripheral muscle weakness. If illness is severe, respiratory muscles become involved, leading to respiratory failure and death unless supportive care is provided. Death occurs in 5-10% of cases of foodborne botulism.

Five categories of botulism are as follows:

1. Foodborne botulism has long been recognized in the United States. It is often associated with home-canned vegetable products, typically, green beans, red beets, peppers, mushrooms, or asparagus. Large outbreaks of botulism have been associated with restaurants using improperly canned foods or mishandled food products. Strict adherence to appropriate processing methods assures the destruction of all bacterial spores and prevention of germination and outgrowth of spores in the product.

2. Wound botulism is the rarest form of the disease. Most cases involve infections in traumatic wounds contaminated with soil, although cases have occurred due to a necrotic bowel or associated with chronic drug abuse. The toxin is elaborated by the organism growing in a wound site.

3. **Infant botulism** is the most common form of the disease in the United States. The disease results from absorption of toxin produced by the toxigenic organisms colonizing the intestinal tract of certain infants under 1 year of age (disease in older toddlers is rare). The lack of established normal gut flora that would inhibit the germination of ingested spores and outgrowth of organisms

is believed to be the cause of the disease. Decreased frequency of bowel movements, which may also be a sign of decreased intestinal motility, is an additional risk factor for infant botulism. Honey has been the only implicated food product and the Centers for Disease Control recommend against feeding honey to an infant less than one year old. Samples of house dust and soils around the home have yielded the same toxin type as the organism isolated from the child.

There is a range of clinical presentations in infant botulism from mild illness not requiring hospitalization to sudden death. Constipation is generally the first symptom although it may be overlooked. Infants who are hospitalized usually develop lethargy and mild weakness with feeding difficulties, pooled oral secretions, and an altered cry. The baby eventually becomes floppy, loses head control and may develop classical botulism signs and symptoms.

4. Child or adult-non-foodborne is characterized by the typical signs and symptoms of botulism, but no food vehicle is identified and there is no evidence of wound botulism. It is believed that under certain rare conditions in the intestinal tracts of adults (or children older than 1 year of age) that *Clostridium* is allowed to proliferate and elaborate toxin, which is absorbed into the bloodstream. Rare cases of infant and adult botulism have been confirmed to be the result of intestinal colonization by non-botulinum species that produce botulinum neurotoxin.

5. Intentional toxin release or Laboratory accident can result in an aerosol exposure to botulinum toxin. In an inhalation exposure, respiratory failure may occur in a much shorter time period. The onset of symptoms extends from 24 hours to 2 days depending on the degree of exposure. The symptoms are similar, presumably without the gastrointestinal symptoms.

COXIELLA BURNETII

Coxiella burnetii is a gram-negative pleomorphic coccobacillus that is obligately intracellular and 0.3 to 0.7 μm in length. *C. burnetii* is the causative agent of the zoonotic disease, Q fever. There is a sporelike form, the small cell variant, which is remarkably stable in extracellular environments. A large cell variant also exists that is the vegetative, metabolically active form. Cattle, sheep and goats are the primary reservoirs of *C. burnetii*. The organisms are resistant to heat, drying and many common disinfectants. Infection of humans usually occurs by inhalation of these organisms from air that contains airborne barnyard dust contaminated by dried placental material, birth fluids and excreta of infected herd animals. Humans are very susceptible to the disease and very few organisms are required to cause infection.

Only about one-half of all people infected with *C. burnetii* show signs of clinical illness. Most cases of Q fever begin with sudden onset of one or more of the following: high fevers, severe headache, general malaise, myalgia, confusion, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhea, abdominal pain, and chest pain. The incubation period is usually 2-3

weeks. In general, most patients will recover to good health within several months without any treatment. Only 1% to 2% of people with Q fever die of the disease. A vaccine for Q fever has been developed and has successfully protected humans in Australia, however, it is not commercially available in the United States.

FRANCISELLA TULARENSIS

F. tularensis is a tiny, pleomorphic, coccobacillus and the etiologic agent of tularemia. Tularemia is primarily a disease of wild animals and is carried by many species of wild rodents, rabbits, beavers, and muskrats. Human infection is incidental and is usually the result of interaction with wild animals.

Tularemia often starts with the sudden onset of flu-like symptoms following an incubation period of 1-21 days. As few as 10-50 organisms will cause disease if inhaled or injected intradermally. The ulceroglandular form of the disease constitutes about 45-85% of the reported cases. This is most often acquired through inoculation of the skin or mucous membranes. Other forms of the disease are: oculoglandular, septic, oropharyngeal, glandular, typhoidal, gastrointestinal, and pneumonic. Inhalation results in typhoidal tularemia, it manifests as fever, prostration, and weight loss, but without lymphadenopathy. Pneumonic tularemia is an illness characterized primarily by pneumonia. It is seen in 30-80% of the typhoidal cases and in 10-15% of the ulceroglandular cases.

***STAPHYLOCOCCUS* ENTEROTOXIN B**

Staphylococcus Enterotoxin B (SEB) is the enterotoxin that most commonly causes classic food poisoning. SEB also causes nonmenstrual toxin shock syndrome. In addition, it has been studied as a biological weapon, because of its stability and the fact that it can be easily aerosolized.

Patients may exhibit a sudden onset of symptoms 1-8 hours after **ingestion** of the enterotoxin. Symptoms include nausea, vomiting, abdominal cramping, diarrhea, dehydration, hypotension, tachycardia and hyperperistalsis. **Inhalation** of the aerosolized form of SEB results in symptoms occurring from 3 to 12 hours after exposure. Symptoms include an abrupt onset of high fever (103 to 106ºF) that lasts for 2 to 5 days, chills, headache, myalgia, and non-productive cough persisting for up to 4 weeks.

VARIOLA VIRUS (SMALLPOX)

Variola virus, the etiologic agent of Smallpox, belongs to the family *Poxviridae***.** The poxviruses are ovoid, brick-shaped particles with rounded corners. They have a dumbbell-shaped core and a lateral body filling the concavity between the outer envelope and the bar of the dumbbell. This is visualized by electron microscopy.

AGENTS OF BIOTERRORISM
GENERAL ORGANISM INFORMATION

Variola is primarily transmitted by inhalation of aerosol droplets. A person becomes infectious after a rash appears and is infectious until the last smallpox scab falls off. The first symptoms of smallpox include fever, malaise, head and body aches and sometimes vomiting. The fever is usually high, in the range of 101 to 104 degrees. A rash emerges first as small red spots on the tongue and in the mouth. Within 24 hours a rash appears on the skin, starting on the face and then spreading to the arms and legs, then the hands and feet. The rash becomes raised bumps that fill with fluid that then become pustules. The pustules then form a crust and then scab.

YERSINIA PESTIS

Yersinia pestis is a non-motile, slow growing, facultative organism, classified in the family *Enterobacteriaceae*. *Yersinia pestis* is a gram-negative bipolar-staining bacillus that often resembles safety pins.

Three forms of plague are recognized in humans:

Bubonic Plague

Typically, plague follows a bubonic course, which is manifested by acute onset 2 to 7 days after exposure and is marked by fever, headache, malaise, myalgia, shaking chills, and pain in the groin, axilla, or neck. Other symptoms may include nausea, vomiting, and diarrhea. At the time of onset, or 1 to 3 days later, a swelling (bubo, thus "bubonic plague") is noted in the affected groin, axilla, or neck; the swelling may become very large, is usually excruciatingly painful, and may interfere with leg, arm, or head movement, depending on location. Untreated, the disease may be rapidly progressive and fulminant. Bacteria are disseminated hematogenously to virtually all organ systems, including the lungs. Untreated bubonic plague is 50% to 60% fatal, but with prompt diagnosis and treatment the number should be near zero.

Septicemic Plague

Approximately 10% of the human plague cases in the United States do not develop external lymphadenopathy. Infections without buboes are termed primary septicemic plague and may be difficult to diagnose. This clinical form is a progressive gram-negative sepsis that, without rapid initiation of specific antibiotic therapy, is often fatal. Septicemic plague may occur in any patient, but most cases have involved either elderly (>55 years) or young (<10 years) patients. The case-fatality rate for primary septicemic plague hovers at about 50%, usually because of delay in achieving a correct diagnosis caused by the lack of specific signs and symptoms.

Pneumonic plague

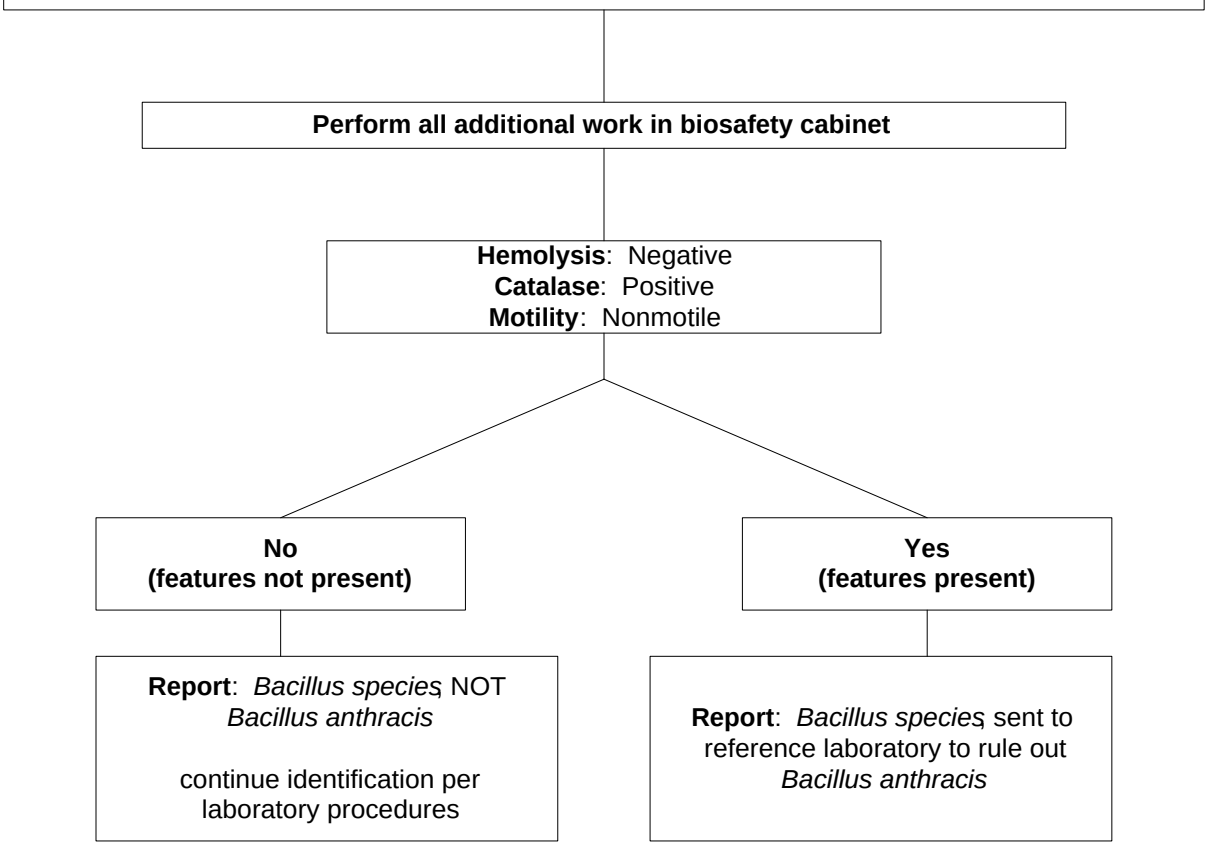
During either bubonic or septicemic courses, as the bacteria progressively invade and destroy the integrity of the alveolar membranes of the lung, organisms mixed with blood, mucus, and sputum may be expelled from the patient via coughing or choking and transmitted to new hosts via airborne droplets. Pneumonia subsequent to bubonic or septicemic plague is termed secondary plague pneumonia. Plague acquired by inhalation of infective droplets is termed primary plague

pneumonia. Primary plague pneumonia may be difficult to separate correctly from pneumonias of other etiologies unless plague is seriously suspected on the basis of known exposure to plague-infected patients or animals. Plague pneumonia is progressive and rapidly fatal unless specific treatment is instituted within about 18 hrs. of onset. It can be so rapidly fatal that persons reportedly have been exposed, become ill and died on the same day. Patients with pneumonic plague may have coughs productive of infectious particle droplets, which are highly contagious.

Bacillus anthracis: Level A Laboratory Flowchart

Morphology: Large aerobic, gram positive rods (1 to 1.5 by 3 to 5 um)
Smears/blood/CSF: Short chains of 2-4 cells that appear encapsulated
Sheep blood agar (ambient atmosphere): Oval, central-to-subterminal spores which do not cause significant swelling of cell; often in long chains

Growth on sheep blood agar: 2-5 mm, tenacious, nonhemolytic colonies after 15-24 hours
(flat/slightly convex, irregularly round colonies with irregular/wavy border and ground glass appearance)



GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *BACILLUS ANTHRACIS*

PURPOSE
The procedures described below function to rule out or presumptively identify *Bacillus anthracis* in clinical specimens or isolates.

SAFETY
All procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL 2) practices and the culture transferred to a certified Class II biological safety cabinet (BSC) as soon as *Bacillus anthracis* is suspected. Laboratory coats and gloves must be worn when processing specimens and performing tests. Safety glasses or eye shields are recommended.

ACCEPTABLE SPECIMENS:
Specimens of choice will be determined by the clinical presentation. ***Environmental or nonclinical samples and samples from announced events are not processed by Level A laboratories; please contact local law enforcement or UDOH directly.***

a. Cutaneous lesions

(1) Vesicular stage: aseptically collect vesicular fluid on sterile swabs from previously unopened vesicles.
NOTE: The anthrax bacilli are most likely to be seen by Gram stain in the vesicular stage.

(2) Eschar stage: collect eschar material by CAREFULLY lifting the eschar's outer edge; insert a sterile swab, then slowly rotate for 2-3 seconds beneath the edge of the eschar without removing it.

Transport directly to laboratory at room temperature. For transport time >1 h and <24 h, transport at 2 to 8°C.

b. **Stool**: Transfer ≥5 grams of stool directly into a clean, dry, sterile, wide-mouth, leak-proof container. Transport unpreserved stool to laboratory within 1 h. For transport time >1h and < 24 h, refrigerate at 2 to 8°C; Cary-Blair or equivalent transport media is acceptable.

c. **Rectal swab**: For patients unable to pass a specimen, obtain a rectal swab by carefully inserting a swab 1 inch beyond the anal sphincter. Transport directly to laboratory at room temperature. For transport time >2 h and <24 h, transport at 4°C.

d. **Blood cultures**: Collect appropriate blood volume and number of sets per laboratory protocol.
NOTE: In later stages of disease (2-8 days post-exposure), blood cultures may yield the organism, especially if specimens are obtained prior to antibiotic treatment. Transport directly to laboratory at room temperature.
Note: Whole blood collected in a purple-top tube may be requested for additional tests.

e. **Sputum**: collect >1 ml of a lower respiratory specimen into a sterile container. Inhalational anthrax usually does not result in sputum formation. Transport in sterile, screw-capped container

at room temperature when transport time is < 1 h. For transport time >1 h and < 24 h, transport at 4°C.

f. **CSF, tissue, autopsy samples** collect aseptically and place in sterile containers. Transport directly to laboratory at room temperature.

g. **Nasal swabs**: See Appendix A at the end of this procedure.

MATERIALS

A. Reagents

1. Gram stain reagents
2. Catalase reagent (3% hydrogen peroxide)
3. Motility media
4. Sterile saline

B. Media

1. 5% Sheep Blood agar (SBA) or equivalent
2. Chocolate agar (CA)
3. MacConkey agar (MAC)
4. Phenyl ethyl alcohol agar (PEA)
5. Blood culture bottles
6. Tubed motility media
7. Tryptic soy broth or equivalent
8. Thioglycollate broth or equivalent

C. Equipment/miscellaneous

1. Blood culture instrument (optional)
2. Light microscope
3. Microscope slides & cover slips
4. Disposable bacteriologic inoculating loops
5. Incubator, 35-37˚C, ambient preferred (CO_2 enriched is acceptable)

SPECIMEN PROCESSING & PRESUMPTIVE IDENTIFICATION

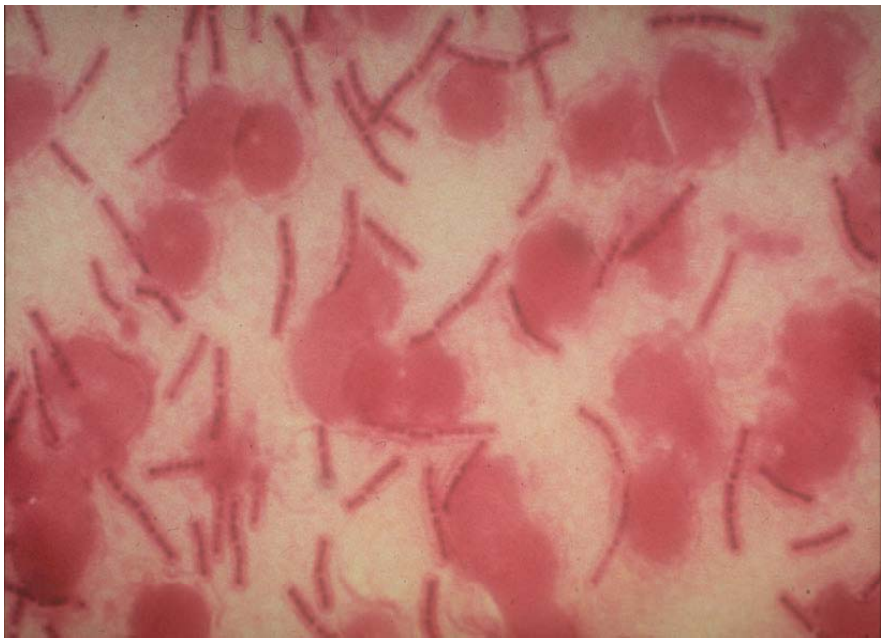
1. Gram Stain

A. Procedure: Perform Gram stain procedure/QC per standard laboratory protocol.

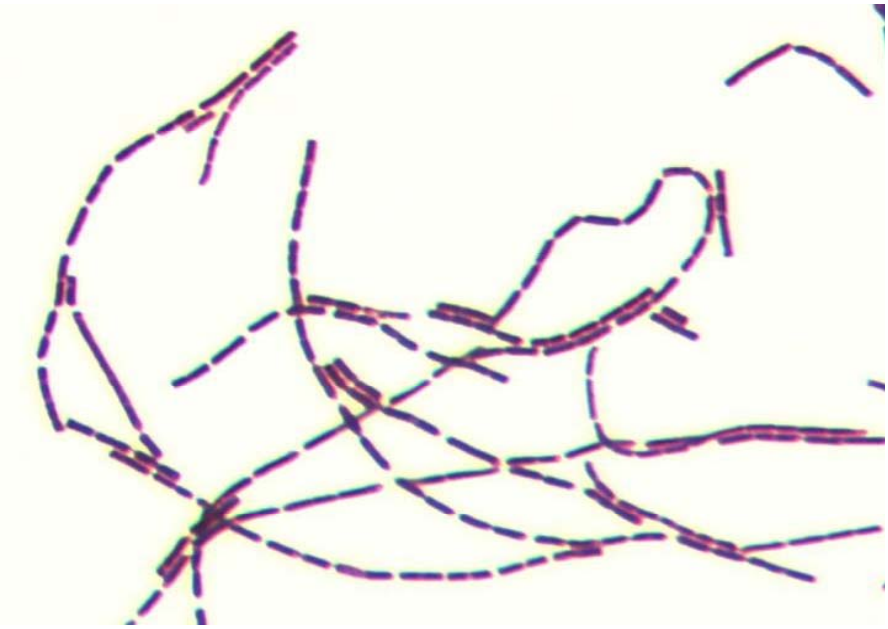
B. Presumptive ID

1. *B. anthracis* is a large gram-positive rod (1-1.5 X 3-5 um)
2. Blood and impression smears: Vegetative cells seen on Gram stain of blood and other clinical smears are in short chains of 2-4 cells that are encapsulated, which may be seen on the Gram stain as clear zones around the bacilli. Spores are not present in clinical samples unless exposed to low CO_2 levels, such as those found in the atmosphere; higher CO_2 levels within the body inhibit sporulation. The presence of large encapsulated gram-positive rods in the blood is strongly presumptive for *B. anthracis* identification.
3. Growth on SBA or equivalent medium: *B. anthracis* forms oval, central-to-subterminal spores on SBA that do not cause significant swelling of the cell; frequently occur as long chains of bacilli. However, cells from growth on SBA are not encapsulated.

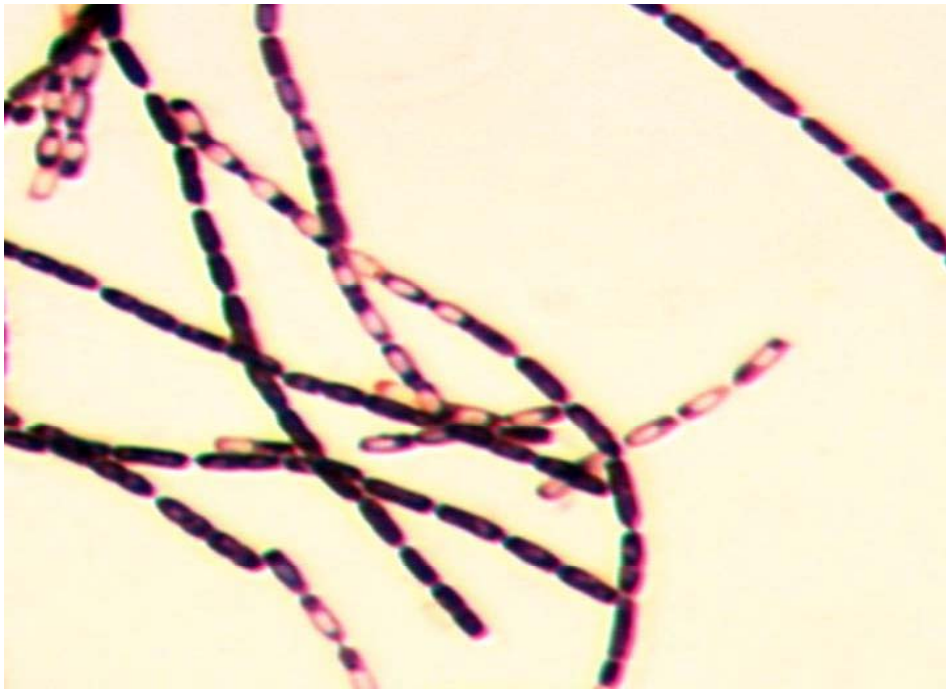
Gram stain of *B. anthracis* in rhesus monkey blood



Gram stain of *B. anthracis* from SBA, magnification 1000X



Gram stain of *B. anthracis* with spores, magnification 1000X.



2. Cultures

A. Inoculation & plating procedure: Inoculate and streak the following media for isolation of the respective specimen types. Note: Standard media should be used according to normal laboratory procedures.

1. Blood cultures: Process following routine laboratory protocol.
2. Cutaneous swab specimens: Plate directly on media used routinely for surface wounds such as SBA, MAC, and broth enrichment, and prepare smears for staining. Note: *B. anthracis* does not grow on MAC.
3. Stool: Plate directly on appropriate media, such as PEA, SBA and MAC.
4. Sputum specimens: Plate directly on media used routinely, such as SBA, MAC and CA, and prepare smears for staining.

B. Incubation:

1. Temperature: 35-37˚C
2. Atmosphere: Ambient preferred
3. Length of Incubation: Hold primary plates for 3 days; read daily. Examine plates with 18-24 hours of incubation. Growth of *B. anthracis* may be observed as early as 8 hours after incubation.

C. Colony characteristics of *B. anthracis*

1. After incubation of SBA plates for 15-24 hours at 35-37˚C, well-isolated colonies of *B. anthracis* are 2-5 mm in diameter. The flat or slightly convex colonies are irregularly round, with edges that are slightly undulate (irregular, wavy border), and have a ground glass appearance. There may often by comma-shaped projections from the colony edge, producing the "medusa-head" colony.

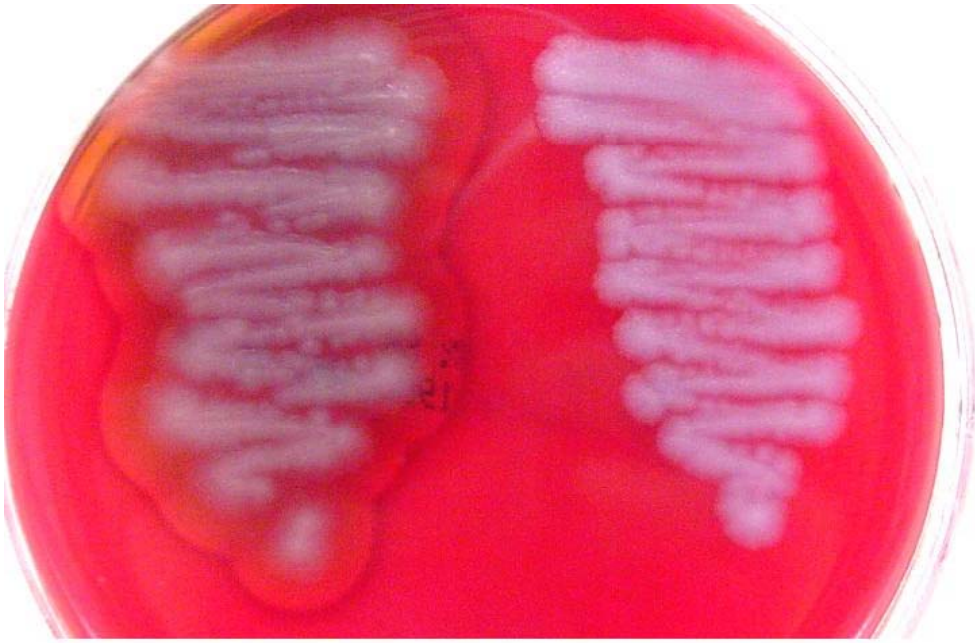
2. *B. anthracis* colonies on SBA usually have a tenacious consistency. When teased with a loop, the growth will stand up like beaten egg whites. In contrast to colonies of *B. cereus* and *B. thuringiensis*, colonies of *B. anthracis* are not β-hemolytic. However, weak hemolysis may be observed under areas of confluent growth in aging cultures and should not be confused with β-hemolysis.
3. When examining primary growth media, it is important to compare the extent of growth on SBA plates with that on MAC. *B. anthracis* grows well on SBA but does not grow on MAC or PEA.
4. *B. anthracis* grows rapidly; heavily inoculated areas may show growth with 6-8 hours and individual colonies may be detected within 12-15 hours. This trait can be used to isolate *B. anthracis* from mixed cultures containing slower-growing organisms.

D. Extent of Identification: For the Level A Laboratory, identification is limited to 'presumptive' only.

B. anthracis colony morphology; overnight cultures on SBA

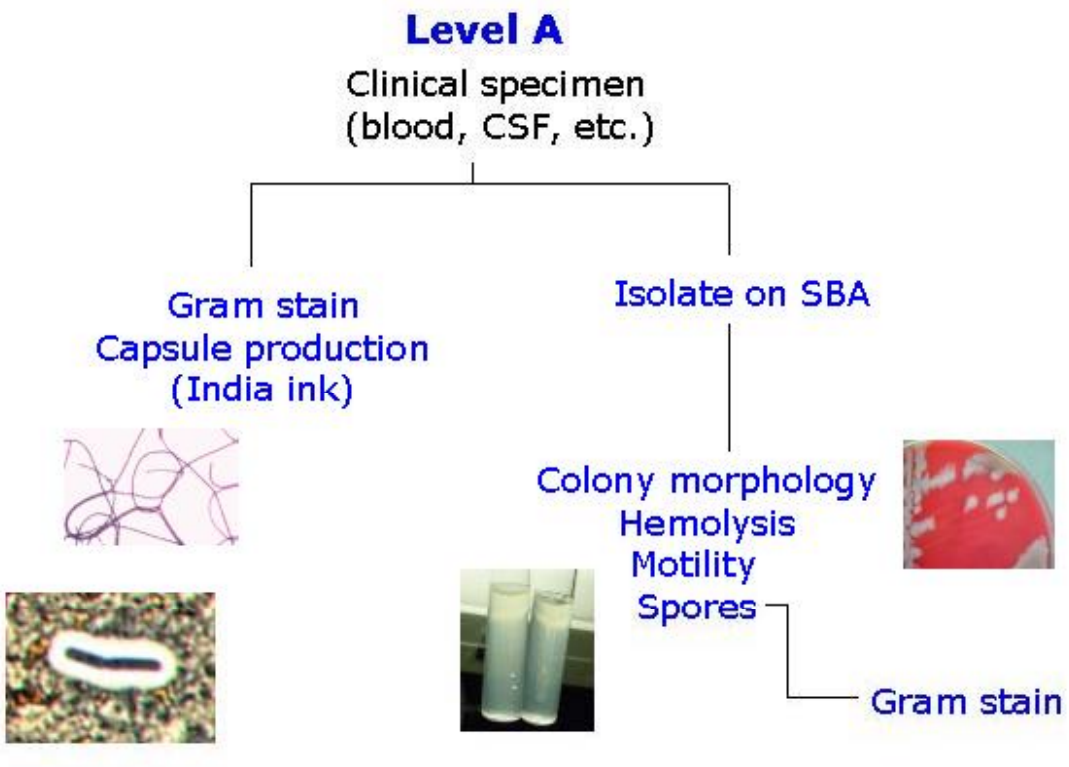
Tenacious colonies of *B. anthracis* on SBA.

B. anthracis and *B. cereus* colony morphology; overnight cultures of *B. cereus* (left side of plate) and *B. anthracis* (right side) on SBA



3. **Motility Test**
   - **A. Purpose:** Used to determine motility of suspected isolates; *B. anthracis* is non-motile.
   - **B. Motility Medium test procedure**
     1. Using a sterile inoculating needle, remove a portion of growth from an isolated, suspect colony after 18-24 hours incubation.
     2. Inoculate the motility medium by carefully stabbing the needle 3-4 cm into the medium and then drawing the needle directly back out so that a single line of inoculum can be observed.
     3. Incubate the tube at 35-37˚C in ambient atmosphere for 18-24 hours.
   - **C. Presumptive ID**
     1. Positive result: Motile organisms will form a diffuse growth zone around the inoculum stab.
     2. Negative result: Non-motile organisms, such as *B. anthracis*, will form a single line of growth that does not deviate from the original inoculum stab.

Presumptive identification criteria: *For the Level A laboratory, identification is limited to "presumptive" only.*



PRESUMPTIVE IDENTIFICATION OF *BACILLUS ANTHRACIS*

Clinical specimens

1. **Gram-positive rods** – Gram stain
2. **Capsule production** – India ink stain

Bacterial Isolate

1. **Spore-former** – Gram stain
2. **Colony morphology** – Sheep blood agar (observation)
3. **Non-hemolytic** – Sheep blood agar (observation)
4. **Non-motile** – Motility medium or Wet mount

PRESUMPTIVE IDENTIFICATION CRITERIA

1. Direct smears from clinical samples, such as blood, CSF, or skin lesion (eschar) material: Encapsulated gram-positive rods.
2. From growth on SBA or equivalent media: Large gram-positive rods (may stain gram-variable after 72 hours of culture). Spores may be found in culture, under non-CO_2 atmosphere (but not on direct examination). Spores are non-swelling and oval-shaped.
3. Rapid, aerobic growth and tenacious colonies on sheep blood agar.
4. Catalase positive.
5. Non-motile: In addition to *B. anthracis*, *B. cereus* var. mycoides is non-motile.
6. Non-hemolytic on SBA, ground-glass appearance of colonies.

RULE OUT

While hemolysis, gram stain morphology, or motility can be used for rule out when the result provides clear evidence that the isolate is not *B. anthracis* (e.g., a clearly visible zone of beta hemolysis), a combination of two Level A tests is recommended for rule out.

ACTIONS IF A *BACILLUS ANTHRACIS* IS SUSPECTED OR PRESUMPTIVELY ISOLATED (REPORTING/ACTION)

1. Correlate laboratory results with clinical findings.
2. As soon as possible, consult with the UDOH and/or Epidemiology; the specimens should be split with one set forwarded to the UDOH for rapid/advanced testing. This is one of the organisms that require that we contact the FBI. However, the isolate needs to be confirmed prior to that notification If *Bacillus anthracis* is used as a bioterrorism agent in a covert or "unannounced" event, immediate and appropriate notification will help save others in the community.

- Emergency #: Pager: 888-EPI-UTAH

- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**

- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: **rrolfs@utah.gov**

3. Package specimens and/or isolate according to shipping requirements (refer to shipping requirement section).
4. Refer original specimen and/or isolate to the UDOH Laboratory (Level B/C Laboratory) for confirmation.
5. Preserve original specimen pursuant to a potential criminal investigation.
6. Assist local law enforcement efforts in conjunction with guidance received from the UDOH.
7. If *B. anthracis* is ruled out, proceed with efforts to identify using established laboratory procedures.

REFERENCES

1. CDC and ASM. Basic Protocols for Level A Laboratories. www.asmusa.org, www.bt.cdc.gov
2. Balows et al. 1988. Laboratory Diagnosis of Infectious Diseases. Springer-Verlag. New York, NY
3. Eitzen et al. 1998. Medical Management of Biological Causalities – Handbook. U.S. Army Medical Research. Fort Detrick, Frederick, MA
4. Murray et al. 1999. Manual of Clinical Microbiology 7th Edition. ASM Press. Washington, D.C.
5. Weyant et al. 1999. Laboratory Protocols for Bioterrorism Response Laboratories for the Identification of *Bacillus anthracis*. APHL. www.aphlnet.org

Appendix A: Nasal specimens for *Bacillus anthracis* screening

PURPOSE: Nasal specimens (nares culture) should ONLY be used to support a confirmed exposure to *B. anthracis* or during an ongoing epidemiologic investigation. Gram stain of nasal specimens for *B. anthracis* is not recommended.

LIMITATIONS: Nasal cultures taken to evaluate for the presence of anthrax spores have not been evaluated for sensitivity or specificity. Nasopharyngeal and throat specimens are not recommended for anthrax screens and should not be submitted. Nasal cultures are NOT recommended for screening those who are asymptomatic and without known exposure.

MATERIALS
Swab (Dacron, rayon or other synthetic swabs are preferred over cotton)
Transport medium for culture.

PROCEDURE

A. Selection

1. The specimen of choice is a swab specimen taken at least 1 cm inside the nares.
2. Lesions in the nose require samples from the advancing margin of the lesions.

B. Method

1. Carefully insert the moistened swab (saline, sterile water) at least 1 cm into the nares.
2. Firmly sample the inside of the nares by rotating the swab and leaving it in place for 10-15 seconds.
3. Withdraw the swab, insert it into its transport container, and submit the sampling unit to the Level A Laboratory for culture.

C. Transport

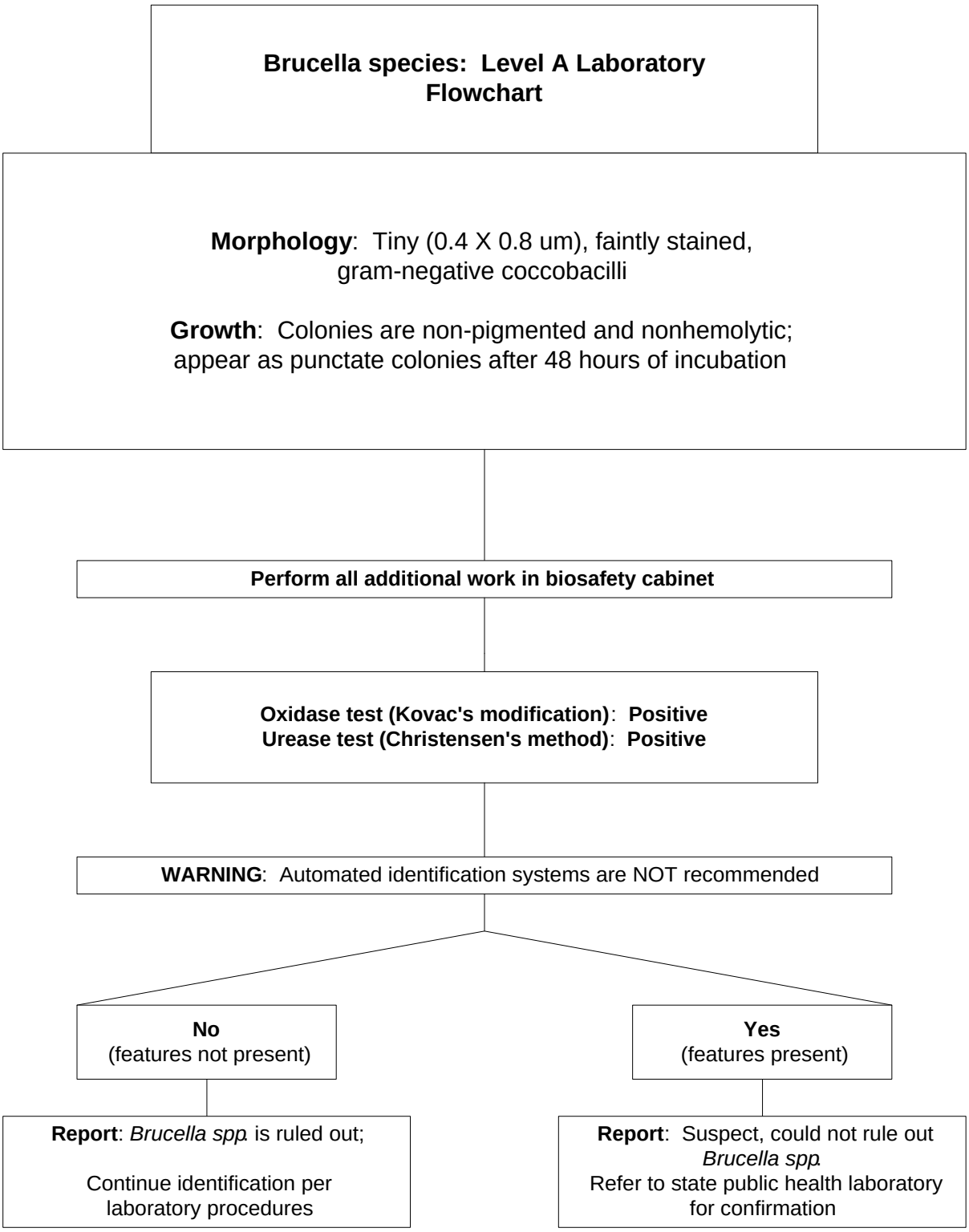
1. Transport the specimen to the Level A Laboratory as soon as possible.
2. Do not refrigerate specimens for culture.

D. Culture: Heat Shock

1. Remove the swab from transport container and place it into 1.5 mL of sterile saline, water or a nutrient broth such as trypticase soy broth, brain heart infusion broth or equivalent. Vigorously twist the swab, and recap the tube.
2. Leave the swab in the tube. Place the broth suspension into a 65˚C water bath for 30 minutes.
3. Plate 100-200 µl of broth on 5% sheep blood agar plate and incubate at 35-37˚C for 18-24 Hours. *Bacillus anthracis* will have visible growth in 12-18 hours; observe for characteristics of *B. anthracis*.

INTERPRETATION: Observe colony morphology for typical *Bacillus* colonies, look for lack of hemolysis, perform Gram stain, and evaluate for *B. anthracis* characteristics as described in the above *B. anthracis* protocol.

REPORTING: If *B. anthracis* cannot be ruled out, submit the isolate to the UDOH Lab for confirmation. Refer to the protocol above for further reporting information.


Brucella species: Level A Laboratory Flowchart
Morphology: Tiny (0.4 X 0.8 um), faintly stained, gram-negative coccobacilli
Growth: Colonies are non-pigmented and nonhemolytic; appear as punctate colonies after 48 hours of incubation
Perform all additional work in biosafety cabinet
Oxidase test (Kovac's modification): Positive
Urease test (Christensen's method): Positive
WARNING: Automated identification systems are NOT recommended
No
(features not present)
Yes
(features present)
Report: Brucella spp. is ruled out;
Continue identification per laboratory procedures
Report: Suspect, could not rule out Brucella spp.
Refer to state public health laboratory for confirmation

GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *BRUCELLA* SPECIES

PURPOSE

The procedures described below function to rule out or presumptively identify *Brucella* spp. from clinical specimens or isolates.

SAFETY

All procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL 2) practices and the culture transferred to a certified Class II biological safety cabinet (BSC) as soon as *Brucella* is suspected. Laboratory coats and gloves must be worn when processing specimens and performing tests. Safety glasses or eye shields are recommended. Specimens with suspected *Brucella* spp. should be labeled as such. **Due to the highly infectious nature of this organism and its visual similarity to *Haemophilus influenzae*, the technique of smelling culture plates SHOULD NOT be used!**

ACCEPTABLE SPECIMENS: ***Environmental/nonclinical samples and samples from announced events are not processed by Level A Laboratories; please contact local law enforcement or the UDOH directly.***

1. **Blood or bone marrow** – these are the sources from which *Brucella* spp. is most often isolated. Standard blood culturing systems. Transport at room temperature.
   Note: Whole blood collected in blue, purple or green top tubes may be requested for additional tests.

2. **Serum** – for serologic diagnosis, an acute phase specimen should be collected as soon as possible after onset of disease. A convalescent phase specimen should be collected >14 days after the acute specimen. Preferably send at least 1 mL, refrigerated.

3. **Spleen, liver, or abscess** – *Brucella* spp. are occasionally isolated from these sources. Selective media can be used for isolation of *Brucella* spp. from specimens with mixed flora (see below). Specimens should be refrigerated (2-8° C) until inoculation.
   Tissue must be kept moist, add several drops of sterile saline if necessary.

MATERIALS

A. Reagents

1. Gram stain reagents
2. Oxidase reagent
3. Urea agar (Christensen's)

B. Media

1. General nutrient agar: 5% Sheep blood agar (SBA), tryptic soy agar base, or equivalent
2. Chocolate agar (CA), Thayer Martin agar or equivalent
3. MacConkey agar (MAC)
4. Blood culture bottles

C. Equipment / Supplies

1. Blood culture instrument (optional)
2. Light microscope
3. Microscope slides, disposable inoculating loops

SPECIMEN PROCESSING & PRESUMPTIVE IDENTIFICATION

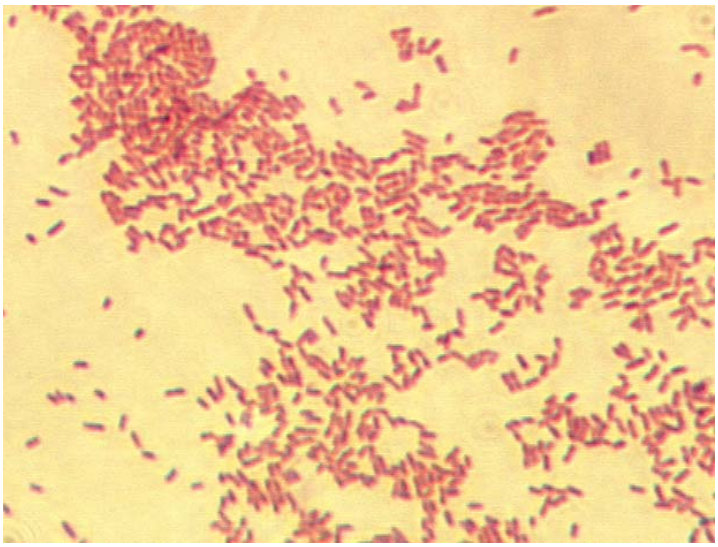
All inoculation procedures and manipulation of possible cultures for *Brucella* species should be performed, while wearing gloves, in a biological safety cabinet.

1. Gram stain

A. Procedure – Perform Gram stain procedure/QC per standard laboratory protocol

B. Presumptive ID

1. *Brucella* spp. have a characteristic Gram-stain morphology that is extremely helpful in differentiating them from other gram-negative organisms. *Brucella* cells appear as tiny, gram-negative coccobacilli.



2. Culture

A. Inoculation & Plating Procedure

Blood cultures, bone marrow and tissue specimens: Process and subculture using standard laboratory protocol to 5% Sheep blood agar, Chocolate agar, MacConkey agar and/or Thayer-Martin agar. Growth on MAC will be negative or poor for most *Brucella* spp. Thayer-Martin can be used as a selective medium for *Brucella* spp. as appropriate.

B. Incubation

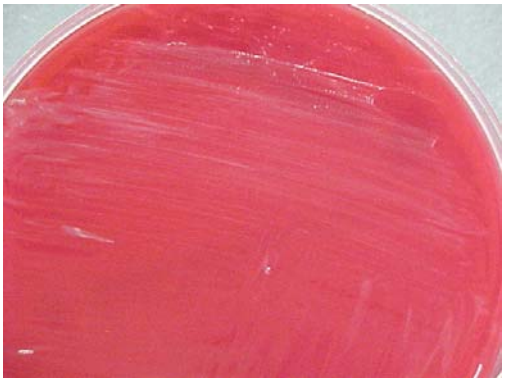
1. Temperature: 35-37˚C
2. Atmosphere: CO_2 enriched, incubator humidity should be sufficient to prevent plates from drying out with prolonged (>7 days) incubation. Humidity may also be maintained by wrapping the plates with gas permeable tape.
3. Length of incubation:
   a. Blood cultures: For suspected Brucellosis cases at least 21 days, with blind sub-culturing every 7 days. Follow by terminal subculturing of negative blood cultures and holding sealed plates for 7 additional days.
   In blood cultures, *Brucella* isolation is often delayed compared to other blood stream pathogens, with peak isolation occurring at 3 to 4 days. With the BACTEC 9240, a

maximal incubation time of 10 days is sufficient for reliable recovery of the organism. For the BactiAlert system, terminal subcultures should be performed at 7 days to increase yield. Lysis-centrifugation has shown to be less sensitive than broth-based systems.

b. Primary plated cultures: 7 days, read daily

D. Colony characteristics of *Brucella* spp

1. *Brucella* colonies typically show "dust-like" growth after overnight incubation, and a minimum of 48 hours is necessary to get sufficient growth for further identification of the organism. The colonies will appear as punctate colonies after 48 hours of incubation in 5 to 10% CO_2 on chocolate or blood agars. Colonies are non-pigmented and non-hemolytic.
2. The organism does not grow on MAC; this will allow it to be separated from some other Gram-negative coccobacilli.
3. Colonies are smooth, convex, and raised with an entire edge (i.e. they have no distinguishing features). All suspicious colonies should be examined by Gram stain, Oxidase, and urea test.



24 and 72 h growth on blood agar

3. Biochemical tests

A. Oxidase test

1. Principle: Used to detect the presence of Oxidase enzymes associated with the Cytochrome respiratory system. The reagent is a dye that changes color in the presence of oxidase enzymes.
2. Specimen: Performed on samples of actively growing colonies from SBA or equivalent plates.
3. Reagents & Materials
   a. Oxidase reagent
   b. Whatman #1 filter paper or equivalent
   c. Disposable plastic loop
4. Procedure (can be performed using other validated laboratory procedure)
   a. Place 1 to 2 drops of Oxidase reagent on a piece of filter paper.
   b. Using a disposable plastic loop, mix a loopful of organisms from a plated culture into the reagent on the paper.
   c. Observe for the development of a light - dark blue color within 10 sec. of inoculation.

5. Characteristics
   a. Positive result: Development of a blue color within 10 sec. of inoculation.
   b. Negative result: No development of blue color.
   c. Some metals in bacteriological loops produce false positive reactions.

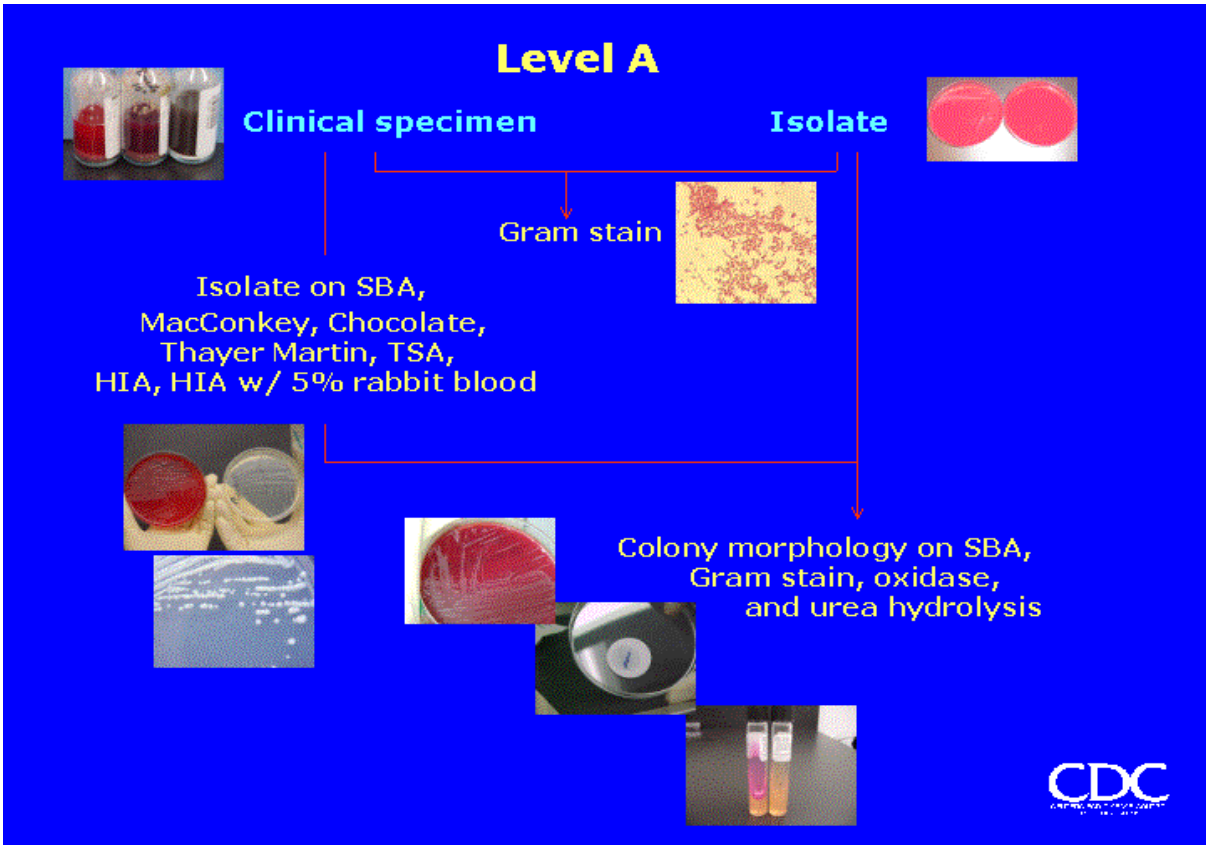
B. Urease test (Christensen's method)

1. Principle: Used to determine an organism's ability to hydrolyze urea, forming ammonia by action of the enzyme urease. The presence and rate of urease activity are useful in the differentiation of *Brucella* spp.
2. Specimen: Actively growing culture of the test organism from non-selective media.
3. Reagents & Materials
   a. Urea agar
   b. Timer
   c. Sterile inoculation loop
4. Procedure
   a. Allow the urea agar to equilibrate to room temperature.
   b. Using a sterile inoculating loop, transfer a loopful of test organism to the surface of the urea agar slant.
   c. Incubate at 35-37˚C in ambient atmosphere.
   d. Set timer for 15 minutes.
   e. After 15 minutes of incubation, observe the inoculated slant for a color change to pink in the inoculated area. If no color change is observed, replace the slant in the incubator and observe it again after 24 hours of incubation.
5. Characteristics
   a. Positive result: Development of a pronounced pink color in the agar. All *Brucella* spp. should produce a positive reaction after overnight incubation.
   b. Negative result: Absence of pink color.
6. Limitations of the procedure: Other gram-negative organisms (*Bordetella bronchiseptica* and some *Haemophilus influenzae* biogroups) may produce a rapid urease reaction. Gram-stain, X and V growth factor requirements and motility are useful characteristics for ruling out these other species. *Brucella* spp. are non-motile.

PRESUMPTIVE IDENTIFICATION CRITERIA

1. Colony morphology on SBA: *Brucella* spp. will appear as punctate colonies after 48 hour incubation. Colonies are non-pigmented and non-hemolytic. All suspicious colony types should be examined by Gram stain and urea test.
2. Gram stain morphology: *Brucella* spp. have a characteristic Gram stain morphology that is extremely helpful in differentiating them from other gram-negative organisms. *Brucella* cells appear as tiny, faintly stained coccobacilli.
3. Oxidase test (Kovac's modification) positive.
4. Urease test (Christensen's method) positive.

Presumptive Identification Criteria: For *the Level A laboratory, identification is limited to "presumptive" only.*



Major Characteristics

1. Tiny faintly stained Gram negative coccobacilli
2. Colonies on SBA will appear as non-pigmented, non-hemolytic, punctate colonies after 48 h incubation. All suspicious colonies should be examined by Gram stain and urea test.
3. Growth in aerobic blood culture bottles take 2 to 4 days
4. No growth on MAC
5. Oxidase positive
6. Urease positive (small numbers are delayed up to 72 hrs)
7. X and V factors NOT required
8. Catalase positive: (This test is NOT recommended if Brucella is suspected because of the generation of aerosols; this test MUST be performed in a BSC if it is performed.)

Differentiation of *Brucella* spp. from similar gram-negative bacteria

Test	*Oligella*[a] spp.	*Haemophilus influenzae*[b]	*Francisella tularensis*	*Brucella* spp.	*Acinetobacter* spp.	*Psychrobacter phenylpyruvicus*[c]	*Pasteurella* spp.	*Bordetella bronchiseptica*[d]
Specimen Source	urinary tract	various	v	blood, bone marrow	various	various	various	various
Gram stain morphology	tiny cocco-bacilli	small coccobacilli	tiny coccobacilli	tiny cocco-bacilli	broad coccobacilli	broad coccobacilli	medium size rods	small to medium rods
Oxidase	+	v	-	+[e]	-	+	+	+
Urea hydrolysis[f]	+	v	-	+	v	+	v	+

[a] *Oligella* spp. can sometimes be motile.
[b] *Haemophilus* spp. require X and/or V factors for growth, which differentiates them from all other listed genera.
[c] Formerly *Moraxella phenylpyruvica*
[d] *B. bronchiseptica* exhibits vigorous growth at one day incubation; motile with peritrichous flagella.
[e] Oxidase: *B. abortus*, *B. melitensis*, and *B. suis* are all oxidase-positive organisms. *B. canis* isolates may be oxidase-variable.
[f] Urea hydrolysis: most *Brucella* isolates vigorously hydrolyze urea. Using the Christensen's tube test, hydrolysis can be observed in as early as 15 min incubation with *B. suis* strains and within 1 day of incubation with most strains of *B. abortus*, and *B. melitensis*.
+ Greater than or equal to 90% positive.
- Less than or equal to 10% positive.
v Variable, 11-89% positive.

LIMITATIONS

1. The identification of *Brucella* species should not be attempted with commercial identification systems because of the potential of generating aerosols and the lack of accuracy in identification.
2. The most common misidentification of *Brucella* is *Haemophilus influenzae* (requires X and V factors). Other organisms that can be confused with Brucella ssp. because they are urease positive are *Oligella ureaolytica* (usually found in the urine), *Psychrobacter phenylpyruvicus, Psychrobacterimmobilis*, and *Bordetella bronchiseptica* (motile).

ACTIONS IF A *BRUCELLA* SPECIES IS SUSPECTED OR ISOLATED

1. Correlate laboratory results with clinical findings.
2. **As soon as possible, contact the UDOH Laboratory and/or Epidemiology; the specimens should be split with one set forwarded to the UDOH for rapid/advanced testing.**

UDOH CONTACT INFORMATION

- **Emergency #: Pager: 888-EPI-UTAH**

- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**

- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: rrolfs@utah.gov

3. Package specimens and/or isolate according to shipping requirements (refer to shipping requirement section).
4. Refer original specimen and/or isolate to the Utah DOH Laboratory (Level B/C Laboratory) for confirmation.
5. Preserve original specimen pursuant to a potential criminal investigation.

REFERENCES

1. CDC and ASM. Basic Protocols for Level A Laboratories. www.bt.cdc.gov, www.asmusa.org
2. Balows et al. 1988. Laboratory Diagnosis of Infectious Diseases. pgs 143-154. Springer-Verlag. New York, NY
3. Eitzen et al. 1998. Medical Management of Biological Casualities – Handbook. U.S. Army Medical Research. Fort Detrick, Frederick, MA
4. Murray et al. 1999. Manual of Clinical Microbiology 7th Edition. ASM Press. Washington, D.C.
5. Weyant et al. 1999. Laboratory Protocols for Bioterrorism Response Laboratories For the Identification of *Brucella spp*. APHL. www.aphlnet.org
6. Weyant et al. 1995. Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria. Williams & Wilkens. Baltimore, MD

GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *BURKHOLDERIA* SPECIES

PURPOSE
The procedures described below function to provide guidance to laboratories for the isolation and preliminary identification of *Burkholderia mallei* and *Burkholderia pseudomallei* from clinical specimens.

SAFETY
B. mallei and *B. pseudomallei* are highly infectious agents and have caused laboratory-acquired infections. If either of these organisms is suspected, observe Biological Safety Level 2 (BSL-2) practices. Laboratory coats and gloves must be worn when processing samples and performing tests. Safety glasses and eye shields are recommended. Perform all work in a biological safety cabinet (BSC).

ACCEPTABLE SPECIMENS: ***Environmental/nonclinical samples and samples from announced events are not processed by Sentinel Laboratories; please contact local law enforcement or the UDOH directly.***

1. **Blood –** Collect blood specimens before antibiotics are administered, when possible. Collect appropriate volume and number of sets per laboratory protocol.

2. **Urine –** Collect a midstream clean-catch specimen or a catherization specimen.

3. **Abscesses, tissue aspirates, fluids –** Collect tissues and fluids rather than swabs, when possible.

4. **Special situations –** Throat, nasal, skin or sputum specimens may be helpful in screening exposed individuals if a release of *B. mallei* or *B. pseudomallei* has been confirmed.

MATERIALS
Required materials are listed within each procedure section. Reagents, culture media and supplies are commercially available unless otherwise indicated.

SPECIMEN PROCESSING & PRESUMPTIVE IDENTIFICATION

A. Isolation of *B. mallei* and *B. pseudomallei*

1. Purpose

To isolate *B. mallei* and *B. pseudomallei* from clinical specimens. Colony morphology on plated media is useful in differentiating *Burkholderia* spp.

2. Culture Media

a. 5% Sheep blood agar (SBA)
b. MacConkey agar (MAC)

c. Brain heart infusion broth (BHI) or trypticase soy broth (TSB)
d. Blood culture bottles
e. *Pseudomonas cepacia* agar (PC)

3. Procedure

Inoculate specimens to culture media and incubate as indicated in Table A-1below.

Table A-1 Isolation media for *B. mallei* and *B. pseudomallei*

Specimen Type	Culture Media	Instructions	Incubation
Abscess, fluids	SBA, MAC, BHI or TSB	Inoculate plates and streak for isolation. Inoculate broth. Prepare Gram stain smear.	Incubate at 35-37°C in air or 5% CO_2 for 96 hours.
Blood	Vented blood culture bottles or blood culture bottle for automated systems.	Subculture at 12-24 hours, 36-48 hours and before final report (for non-automated systems). Organisms may be present before bottles become turbid. Prepare gram stain at time of subculture.	Incubate at 35-37°C in air or 5% CO_2 for at least 7 days.
Skin swab	SBA, MAC, PC	Inoculate plates and streak for isolation	Incubate at 35-37°C in air or 5% CO_2 for 96 hours.
Sputum	SBA, MAC, PC	Inoculate plates and streak for isolation. Prepare Gram stain smear.	Incubate at 35-37°C in air or 5% CO_2 for 96 hours.
Throat or nasal swab	SBA, MAC, PC	Inoculate plates and streak for isolation	Incubate at 35-37°C in air or 5% CO_2 for 24-96 hours.
Urine	SBA, MAC	Use standard protocol	Incubate at 35-37°C in air for 96 hours.

4. Interpretation

Examine culture media DAILY for organisms with growth characteristics listed in Table A-2 below.

B. Colony Morphology

B.mallei and *B. pseudomallei* grow on SBA and usually MAC, but *B. mallei* may grow more slowly than many other gram-negative bacilli.

Table A-2 Growth characteristics of *B. mallei* and *B. pseudomallei*

Culture Media	Organism	Colony Characteristics
SBA	*B. mallei*	Smooth, gray, translucent colonies at 48 hours
SBA	*B. pseudomallei*	Smooth creamy colonies at 48 hours; some may be mucoid and become dry and wrinkled at 48-72 hours.
MAC	*B. mallei*	Light pink colonies at 72 hours or no growth
MAC	*B. pseudomallei*	Pink colonies at 24-48 hours or colorless colonies at 48 hours.
PC	*B. mallei*	Small white colonies at 72 hours.
PC	*B. pseudomallei*	Large white colonies at 24 hours; media changed to pink color
Blood culture bottles	*Burkholderia* spp.	Rarely macroscopically positive in 24 hours, e.g., change in color, turbidity, or appearance of gas. Over 50% are positive on subculture at 12-24 hours.

NOTE: *B. pseudomallei* produces a characteristic earthy odor on plated media after 48 hours of incubation. **DO NOT SNIFF PLATES**. Odor may be present from closed plates as culture ages.

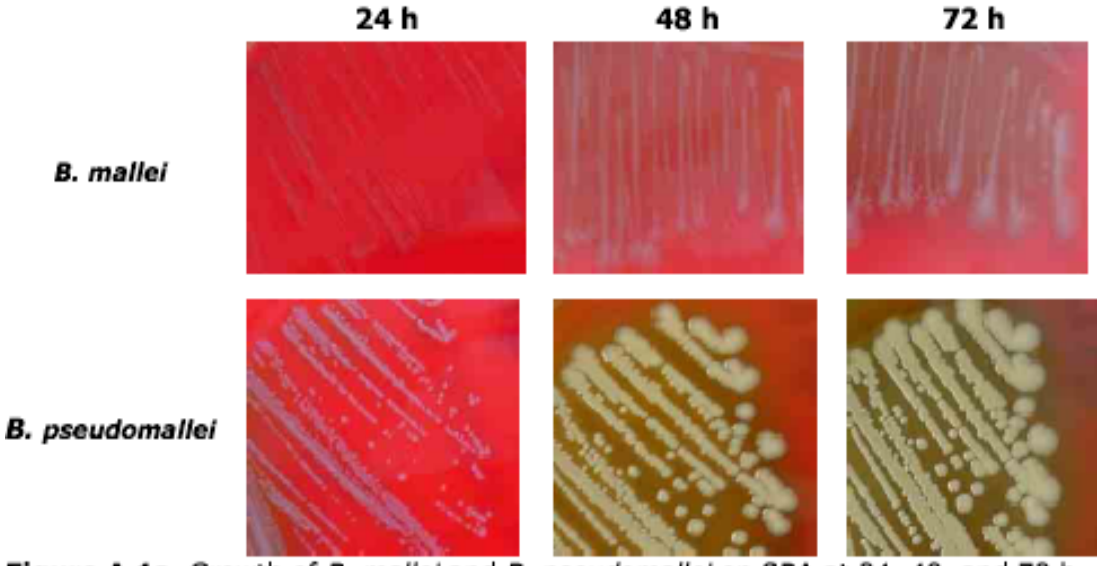


Figure A.1a Growth of *B. mallei* and *B. pseudomallei* on SBA at 24, 48, and 72 h.

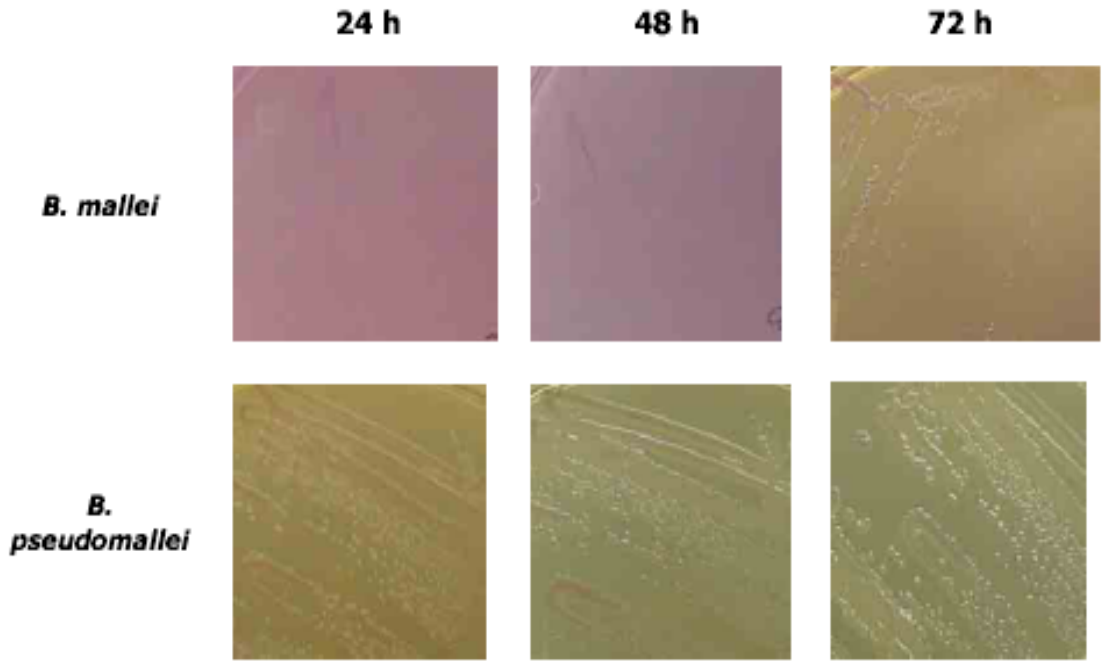


Figure A1.b Growth of *B. mallei* and *B. pseudomallei* on MAC Agar at 24, 48, and 72 h.

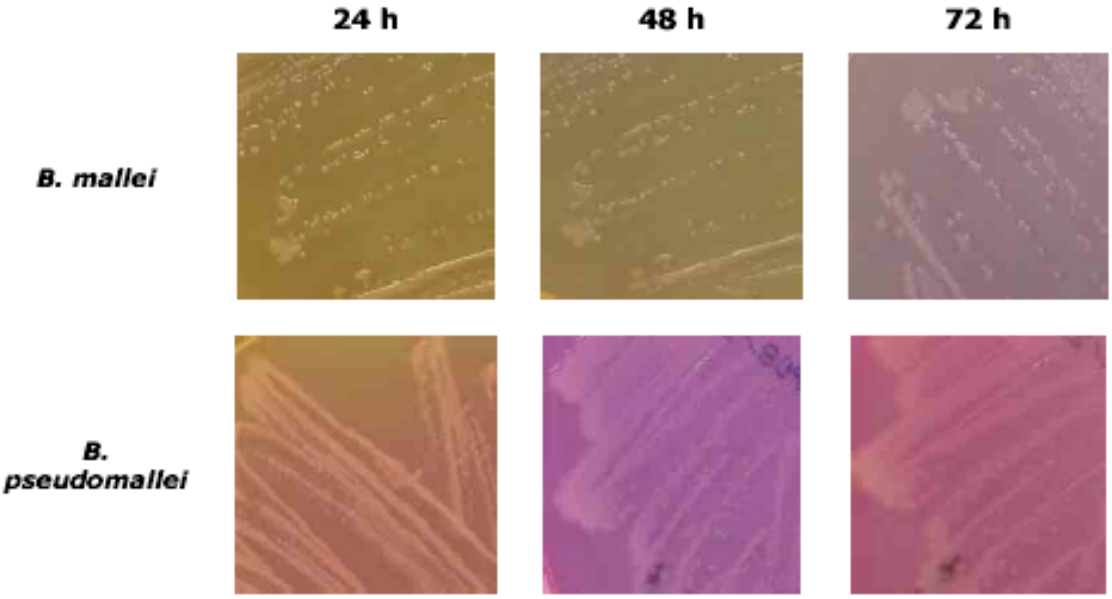
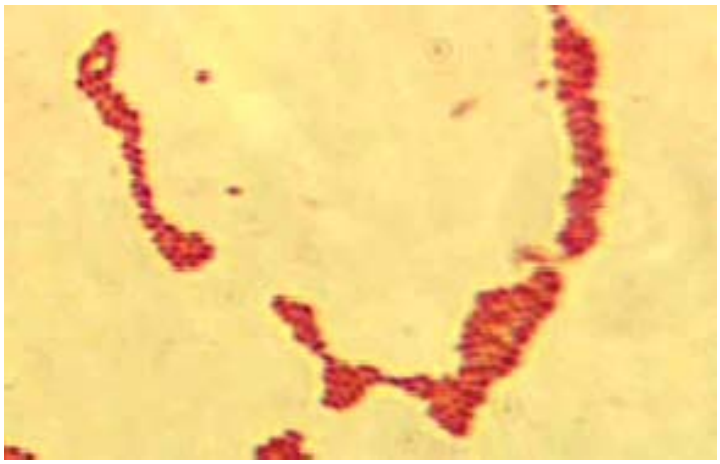


Figure A.1c Growth of *B. mallei* and *B. pseudomallei* on PC Agar at 24, 48, and 72 h.

C. Gram stain morphology

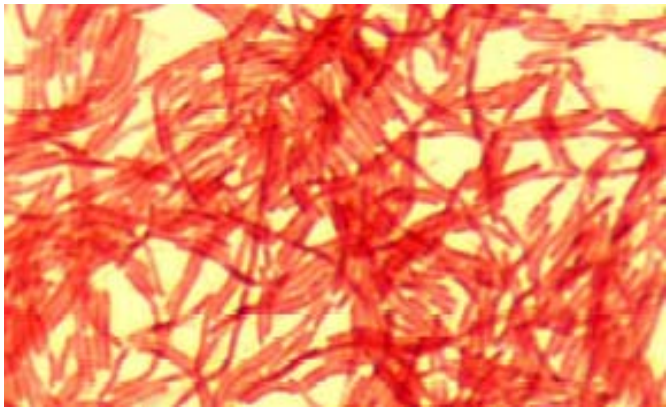
1. **Purpose**
   Observe Gram stain morphology
2. **Materials**
   a. Gram stain reagents
   b. Precleaned glass slides
3. **Procedure**
   a. Prepare smears as follows:
      (1) Clinical specimens
      Prepare thin smear on clean glass slide. Allow to air dry. Heat or alcohol fix smear.
      (2) Blood cultures
      Using syringe or sterile venting unit, apply one drop of broth from the blood culture bottle to a clean slide. Spread with inoculating loop to create uniform smear. Allow to air dry. Heat or alcohol fix smear.
      (3) Colonies from solid media
      Place a drop of sterile saline on a slide. Using inoculating loop or needle, pick a small portion of colony and mix gently in saline. Spread to make a thin smear. Allow to air dry. Heat or alcohol fix smear.
   b. Perform Gram stain per manufacturer's instructions. Blot or air dry.
   c. Examine slide using 100X oil immersion objective on microscope.
4. **Interpretation**
   Observe for characteristic organisms (see below).

   ***B. mallei* –** Faintly staining gram-negative straight or slightly curved coccobacilli (1-3μm) with rounded ends and irregularly parallel or wavy sides. Cells are arranged in pairs end-to-end, in parallel bundles, or in Chinese letter form.



B. mallei

B. pseudomallei – Gram-negative with bipolar staining, 1-3µm in length. Smooth form Appears as long parallel bundles of rods that resemble filaments. Rough form appears more Irregularly arranged.



B. pseudomallei

D. Oxidase test

1. **Purpose**
   To detect the presence of oxidase enzymes associated with the cytochrome respiratory system. The reagent is a dye that changes color in the presence of oxidase enzymes.
2. **Materials**
   a. Oxidase reagent
   b. Whatman #1 filter paper
   c. Petri dish lid
   d. Disposable plastic loop
3. **Procedure**
   a. Perform oxidase test on suspect colonies.
   b. Example procedure: (can be performed using other validated laboratory procedure)
      (1) Place a piece of Whatman #1 filter paper in petri dish lid.
      (2) Drop 1-2 drops of oxidase reagent on filter paper.
      (3) Using a disposable loop, pick suspicious colony and spread on oxidase reagent soaked filter paper. NOTE: Some metal bacteriological loops produce false positive reactions.
4. **Interpretation**
   a. Observe for development of a dark blue to bluish-purple color within 10-30 sec. development of a dark blue to bluish-purple color is considered positive. No change in color is negative. A delayed color development should be ignored.
   b. Compare results to organism characteristics listed below:
      ***B. mallei* – Oxidase variable**
      ***B. pseudomallei* -- Oxidase positive**

E. Motility test

1. Purpose

To determine the motility of isolates.

2. Materials

a. Motility test medium with 2,3,5-triphenyltetrazolium chloride (TTC) indicator.
b. Sterile inoculating loop or needle.

3. Procedure

a. Using a sterile inoculating needle, remove a small portion of growth from an isolated, suspicious colony of an 18-24 hour culture.
b. Inoculate the motility tube by carefully stabbing the center of the medium to ¾ the depth. Draw the needle straight out of the media tube so that a single line of inoculum can be Seen.
c. Inoculate positive and negative (motile and non-motile) control organisms in the same manner as the test organisms. Positive = *Enterobacter aerogenes* 13048. Negative = *Klebsiella pneumoniae* 10031.
d. Incubate the tubes in aerobic atmosphere at 35-37°C for 48 hours.

4. Interpretation

Observe growth in the tube:

a. A diffusible red-colored growth spreading away from the original stab line indicates a positive result (motile).
b. A red-colored growth confined to the original stab line is a negative result (non-motile).
c. Use of an inoculating needle rather than a loop may make the test easier to read. The motility test results shown in the figure below were obtained using a 1-µl loop.
d. Compare results to the organism characteristics below.

***B. mallei* – Non-motile**

***B. pseudomallei* -- Motile**

NOTE: Wet mount motility tests are not recommended due to the potential biohazard they may present.

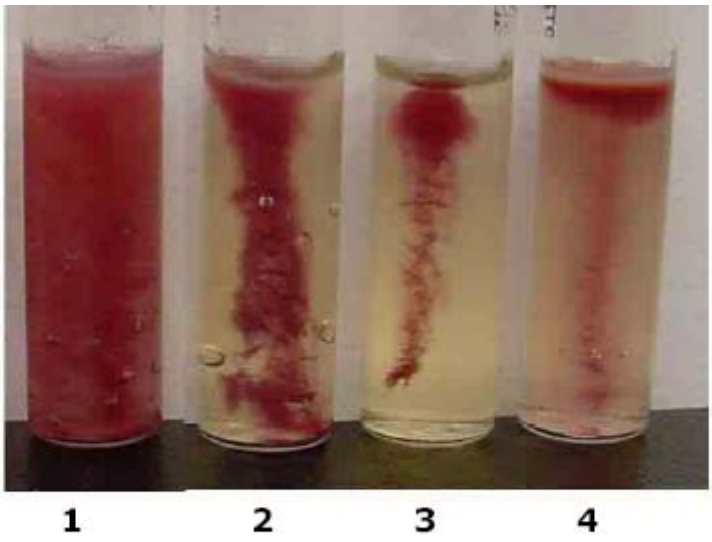


Figure A.3 Motility test in TTC Motility Medium after 48 hrs incubation
Tube 1: *E. aerogenes* ATCC 13048 (positive control) motile
Tube 2: *K. pneumoniae* ATCC 10031 (negative control) non-motile
Tube 3: *B. mallei* (non-motile)
Tube 4: *B. pseudomallei* (motile)

F. Triple sugar iron (TSI)

1. **Purpose**
   To differentiate gram-negative bacilli on the basis of carbohydrate utilization patterns.
2. **Materials**
   a. Triple sugar iron agar slant
   b. Sterile inoculating needle or 1-µl loop
   c. Test tube rack
3. **Procedure**
   a. Using an inoculating needle, pick the center of a well-isolated colony.
   b. Streak the slant and stab to the bottom of the butt with the needle.
   c. Incubate for 24-48 hours at 35-37°C in aerobic atmosphere. DO NOT TIGHTEN CAP.
   d. Examine the color change in the TSI tube to determine the culture's mode of carbohydrate utilization (oxidation, fermentation or non-utilization of carbohydrates).
4. **Interpretation**
   Burkholderia spp. are carbohydrate oxidizers and nonfermenters, thus no change in color of butt is expected. Occasionally, some *Burkholderia pseudomallei* strains (<50%) produce a yellow color on the slant due to oxidation of both lactose and sucrose.

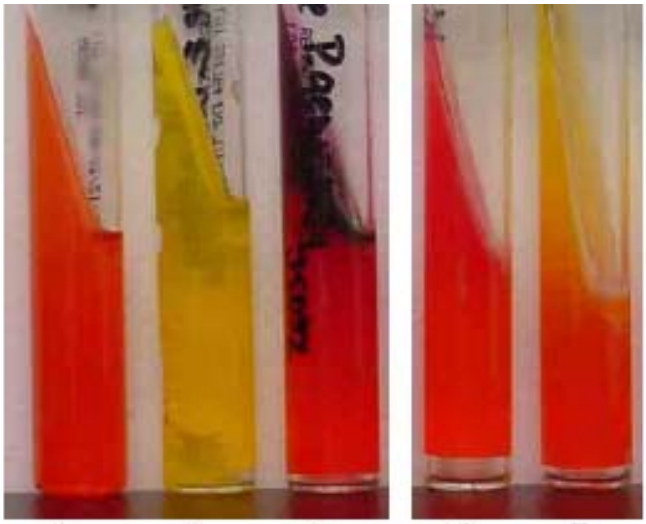


Figure A.4: Carbohydrate utilization in Triple Sugar Iron Agar (TSI) after 24 hours incubation.
Tube 1: Uninoculated tube
Tube 2: *K. pneumoniae* ATCC 10031 (glucose fermenter, positive control)
Tube 3: *P. aeruginosa* ATCC 35032 (non-fermenter, negative control)
Tube 4: *B. mallei*
Tube 5: *B. pseudomallei* (oxidation on slant)

G. Growth at 42°C

1. Purpose

To determine the ability of an isolate to grow at 42°C.

2. Materials

a. SBA plates
b. Sterile inoculating loop
c. Incubator set at 42°C

3. Procedure

a. Inoculate SBA plates with the test organism.
b. Incubate plates at the indicated temperature for 72 hours.

4. Interpretation

Both *B. mallei* and *B. pseudomallei* grow at 42°C on SBA. *B. pseudomallei* produces heavy growth at 48 hours, but *B. mallei* produces only very light growth at 72 hours.

H. Commercial bacterial identification systems

For those laboratories using semi-automated or automated identification systems, care must be exercised when identifications of *B. mallei* or *B. pseudomallei* are obtained. Most manufacturer's have limited data for strains of *B. mallei* or *B. pseudomallei* because they are infrequently identified in the United States.

Due to the low number of strains of *B. mallei* and *B. pseudomallei* in the databases of these identification systems, the opportunity for a misidentification of *B. mallei* or *B. pseudomallei* as another organism must be considered.

If *B. mallei* or *B. pseudomallei* are suspected and a commercial identification system yields Another identification, you should perform assays described in this protocol or submit the Organism to a higher level LRN laboratory for further testing.

INTERPRETATION

B.mallei and *B. pseudomallei* are rarely isolated in the U.S. The one documented case of Glanders in the last 50 years was acquired as a result of a laboratory accident associated with research. Cases of Melioidiosis caused by *B. pseudomallei* have generally been related to travel to endemic areas, such as Thailand. Therefore, the organisms may not be familiar to most of the laboratory staff. In the event of an intentional release of *B. mallei* or *B. pseudomallei*, there will likely be several cases that present with similar organisms. Microbiologists should treat unfamiliar, non-fermenting gram-negative rods with the following characteristics with caution and suspicion.

Suspect level identification criteria for *B. mallei* and *B. pseudomallei*

Characteristic	*B. mallei*	*B. pseudomallei*
Gram stain	Faintly staining gram-negative coccobacilli	Faintly staining gram-negative medium, slender rods (1-3μm) with bipolar staining.
Colony morphology	Light pink colonies on MAC and gray colonies on SBA	Pink or colorless colonies on MAC and white colonies on SBA
Growth at 42°C	No growth at 48 hours, but faint growth at 72 hours.	Heavy growth at 48 hours.
Oxidase test	Variable	Positive
Motility test	Non-motile	Motile
TSI	Butt: Red (no change) Slant: Red	Butt: Red (no change) Slant: Variable

Organisms meeting the above criteria are considered suspect level *B. mallei* or *B. pseudomallei.*

ACTIONS IF *B. MALLEI* OR *B. PSEUDOMALLEI* IS SUSPECTED OR ISOLATED

A. If *B. mallei* or *B. pseudomallei* infection is suspected by a physician, consult the UDOH Laboratory and/or Epidemiology as soon as possible.

B. If an isolate has met the criteria for suspect level identification above, immediately notify the UDOH Laboratory and/or Epidemiology. The UDOH will notify the local FBI, if appropriate.

C. If an isolate has met the criteria for suspect level identification above, immediately notify the physician according to institution's policies.

D. Preserve original specimens pursuant to a potential criminal investigation and transfer to the UDOH Laboratory. Start chain of custody documentation. Follow packaging guidelines for transfer of isolate and/or specimens.

UDOH CONTACT INFORMATION

- **Emergency #: Pager: 888-EPI-UTAH**
- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**
- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: **rrolfs@utah.gov**

REFERENCE

1. CDC and ASM. Basic Protocols for Level A Laboratories. **www.bt.cdc.gov**, **www.asmusa.org**

GUIDELINES FOR SENTINEL LABORATORIES FOR *CLOSTRIDIUM BOTULINUM* TOXIN DETECTION AND CULTURE

PURPOSE

This procedure is for Level A Laboratories and is designed to ensure the proper collection and distribution of appropriate specimens to designated testing laboratories for the testing of *Clostridium botulinum*.

ACCEPTABLE SPECIMENS - *Environmental/nonclinical samples and samples from announced events are not processed by Level A laboratories; please contact local law enforcement.*

Foodborne Botulism

1. Clinical specimens – serum, gastric contents, vomitus, stool, return from a sterile water enema.
2. Autopsy samples – serum gastric and intestinal contents
3. Food samples and/or empty containers with the remnants of the food

Infant Botulism

1. Feces or return from a sterile water enema.
2. Serum – generally not useful since an infant's body mass is small and the toxin is quickly absorbed.
3. Autopsy samples – intestinal contents from different levels of the small and large intestine.
4. Food and environmental (soil and house dust) as appropriate per the investigation.

Wound Botulism

1. Serum
2. Exudate, tissue or swab samples of wound (transported in anaerobic transport media)
3. Isolate of suspect *Clostridium botulinum* submitted in an anaerobic transport vessel.
4. Feces or return from a sterile water enema (wound may not be the source).

Intentional toxin release or Laboratory Accident

1. Serum, Nasal swab
2. Feces or return from a sterile water enema.
3. Food
4. Environmental swabs

MATERIALS

A. Media: Anaerobic media (chopped meat or equivalent); follow standard laboratory protocols.

B. Supplies

1. Port-A-Cul vials or equivalent
2. Leakproof containers (i.e., sealed plastic bags, plastic containers)
3. Petroleum jelly or petrolatum or equivalent (i.e., Vaseline)
4. Sterile, non-bacteriostatic water
5. Packaging materials

PROCEDURE

A. Collection

1. **Feces**: Place into sterile unbreakable container and label carefully. Confirmatory evidence of botulism may be obtained from 10-50 gram quantities (Walnut size); botulism has been confirmed in infants with only "pea-sized" stool samples. The specimen must be kept cool or refrigerated, do not freeze unless an unavoidable delay of several days is anticipated. Freezing does not affect the ability to detect toxin, but does affect the ability to detect the organism.
2. **Enema**: Place approximately 20 ml into sterile unbreakable container and label carefully. If an enema must be given because of constipation, a minimal amount of fluid (preferably non-bacteriostatic water) should be used to obtain the specimen so that the toxin will not be unnecessarily diluted. Transport in a Port-A-Cul vial to maintain anaerobiosis. Specimens must be kept cool or refrigerated.
3. **Gastric aspirate or vomitus**: Place approximately 20 ml into sterile unbreakable container and label carefully. Transport in a Port-A-Cul vial to maintain anaerobiosis. Specimens must be kept cool or refrigerated.
4. **Serum**: Use red top or separator type tubes to obtain serum (no anticoagulant). Samples should be obtained as soon as possible after the onset of symptoms and before antitoxin is given. Enough blood should be collected to provide at least 10 mL of serum (approximately 20 mL of whole blood). Serum volumes less than 3 ml will provide inconclusive results. Whole blood should not be sent as it typically undergoes excessive hemolysis during transit. Specimen should be kept cool or refrigerated, do not freeze unless an unavoidable delay of several days is anticipated.
5. **Tissue, wounds or exudates**: Place into sterile unbreakable container and label carefully. Specimens should be placed in Port-A-Cul vials and sent to the appropriate laboratory, preferably without refrigeration, for attempted isolation of *C. botulinum*. Swabs of superficial wounds are not acceptable for anaerobic culture. Maintain specimens at room temperature.
6. **Postmortem**: Obtain specimens of intestinal contents from different levels of small and large intestines. Place approximately 10 grams per specimen into sterile unbreakable container and label carefully. Obtain gastric content, serum and tissue is/as appropriate. Keep the samples cool or refrigerated.
7. **Culture**: Ship suspicious isolates anaerobically (overlay liquid media with 2-inch layer of sterile petroleum jelly; melt/temper prior to overlaying culture). Cultures may be shipped at room temperature or refrigerated.
8. **Food specimens**: Foods should be left in their original containers if possible, or placed in sterile unbreakable containers and labeled carefully. Place containers individually in leakproof containers (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected foods can be examined. Foods most likely to allow growth of *C. botulinum* will have a pH of 3.5-7.0 (usually 5.5-6.5). Possible foods include:
    - home canned products having a low acidity (pH of 4.6 or greater)
    - foods with low salt or low sugar content
    - foods that are held at temperatures that allow the organism to grow (optimal 35°C, but as low as 15°C)
    - foods that are consumed without prior heating.

Foods that are commercially processed are rarely incriminated; however, the threat to public health is much greater with a commercial foodstuff. Unopened containers are to be sent to the U.S. Food and Drug administration (FDA), with prior arrangement. Keep the samples cool or refrigerated, do not freeze.

9. **Swab samples**:
   a. **Clinical:** Send swabs in an anaerobic transport medium (e.g., Port-A-Cul tubes). For aerosolized botulinum toxin exposure, obtain nasal swabs for culture for *C. botulinum*. For toxin testing, serum should be used. Swabs may be shipped at room temperature or refrigerated.

****Specimens that are frozen must remain frozen until it is time to perform the test.**

B. Transportation - For complete guidelines, refer to packaging and shipping protocol at the end of this manual.

1. If an unavoidable delay of several days is anticipated, the specimens (serum or stool) should be kept frozen and then packed in an insulated container with dry ice and proper cushioning material for shipment. Freezing does not affect the ability to detect botulinum toxin in specimens; freezing does reduce the probability of recovering *C. botulinum*. Since direct detection of toxin provides the best laboratory confirmation of botulism, priority should be given to preserving preformed toxin prior to transport.
2. The receiving laboratory (UDOH Lab) should be notified in advance by telephone as to when and how specimens will be shipped and when they will arrive.

LIMITATIONS

1. If the patient has been taking any medication that might interfere with toxin assays or culturing of the stool, the laboratory should be notified. For example, it has been demonstrated that anticholinesterase drugs given orally to patients for myasthenia gravis can interfere with botulinum toxin assays of stool extracts.

2. Recovery of viable cells from specimens often proves difficult. Proper handling, packaging, and shipping with minimal delay improves recovery. Follow all shipping requirements.

SPECIMEN RELATED INFORMATION

A. Food

1. Foods most likely to allow growth of *C. botulinum* will have a pH range of 3.5 to 7.0, the most common pH is 5.5 to 6.5. However, suspected foods, regardless of pH, can be examined since localized environmental conditions may be present that may support the growth of *C. botulinum.*

B. Feces

1. *C. botulinum* has been isolated from stools following antitoxin treatment. If the patient has been taking any medication that might interfere with toxin assays or culturing of the stool, the laboratory should be notified. A series of two samples may be required to detect infant botulism.

CONFIRMATION INFORMATION

●Foodborne botulism is confirmed by identifying botulinum toxin in samples of suspect food; in the serum of affected patients (within 1-2 days of onset of symptoms); in stools, vomitus, or gastric contents of the patient; or by isolating *C. botulinum* from the patient's stool.

●Wound botulism is confirmed by identifying botulinum toxin in the patient's serum or isolating *C. botulinum* from a wound specimen.

●Infant botulism is confirmed by identifying botulinum toxin in the patient's stool. *C. botulinum* may also be isolated from the patient's stool. Toxin is usually present in relatively high titers (1:100) in the feces during the acute phase of the illness. In the United States, type A toxin is more frequently detected in patients living west of the Mississippi River, whereas, type B predominates in regions east of the Mississippi River.

●Botulism is confirmed by identifying botulinum toxin in the patient's serum or stool or by isolating the organism from the patient's stool.

●Botulism is confirmed by identifying botulinum toxin in the patient's serum.

ACTIONS IF A CLOSTRIDIUM BOTULINUM IS SUSPECTED (*does not apply to infant botulism)*

1. Preserve original specimen pursuant to a potential criminal investigation.
2. Consult with state epidemiologist if *C. botulinum* toxin is suspected.
3. Environmental/nonclinical samples and samples from announced events are not processed by Level A laboratory; submitter should contact local law enforcement directly.
4. Assist local law enforcement efforts in conjunction with guidance received from the UDOH.
5. FBI and state public health laboratory/state public health department will coordinate the transfer of isolates/specimens to a higher-level LRN laboratory as appropriate.

REFERENCES

1. CDC and ASM. Basic Protocols for Level A Laboratories. www.bt.cdc.gov, www.asmusa.org

GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *COXIELLA BURNETII*

PURPOSE
The procedures described below function to rule out or presumptively identify *C. burnetii* in clinical specimens or isolates.

ORGANISM INFORMATION
Since this organism is an obligate intracellular organism, it cannot be cultured on routine bacteriologic media. The laboratory diagnosis of Q fever is based mainly on serologic testing.

SAFETY
Because of the highly infectious nature of this organism, biosafety level 3 requirements are recommended. Specimens from suspected cases of Q fever should be immediately forwarded to the Utah Department of Health Laboratory for identification. Due to the extreme infectivity of *C. burnetii*, sentinel laboratories should not attempt to culture this organism, but should be aware of the potential for inadvertent isolation of *C. burnetii* in cell culture systems designed for virus isolation.

Special decontamination procedures are necessary for surfaces potentially contaminated with *C. burnetii*. Household bleach solutions may be ineffective. Minor spills should be covered with absorbent paper, and then flooded with 70% ethanol or 5% MicroChem-Plus (a quaternary ammonium compound), which should be allowed to act for 30 minutes before cleanup.

ACCEPTABLE SPECIMENS
Note: Sentinel laboratories should not accept environmental/non-clinical specimens. These specimens should be forwarded directly to the Utah Department of Health Laboratory. If a bioterrorism event is suspected, please contact local law enforcement.

A. Serum: Collect serum (red-top or serum separator tube, tiger-top tube) as soon as possible after onset of symptoms (acute phase) and with a follow-up specimen (convalescent phase) at $\geq$ 14 days for serological testing.

B. Blood: Collect blood in EDTA (lavender) or sodium citrate (blue) vacutainer tubes and maintain at 4°C for storage and shipping for PCR or special cultures. If possible, collect specimens prior to antimicrobial therapy.

C. Tissue, body fluids, nasopharyngeal swabs, tracheal/bronchial washings, lesion exudates: Specimens can be kept at 2-8°C if transported within 24 hours. Store frozen at -70°C or on dry ice.

D. Bacterial isolates

ACTIONS IF *COXIELLA BURNETII* IS SUSPECTED.

1. Correlate lab results with clinical findings.
2. **As soon as possible contact the UDOH Laboratory and/or Epidemiology.**

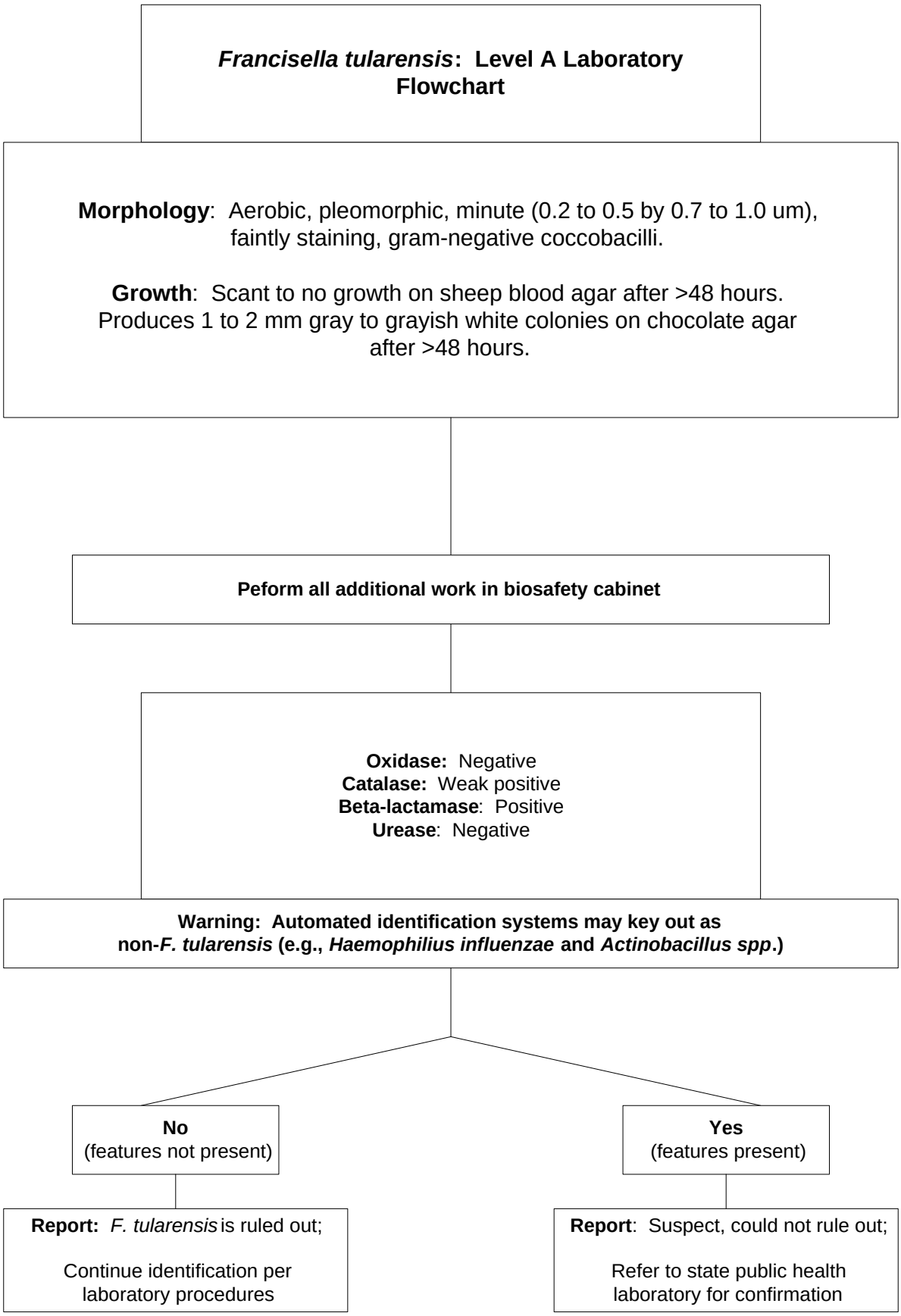
UDOH CONTACT INFORMATION

- **Emergency #: Pager: 888-EPI-UTAH**
- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**
- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: **rrolfs@utah.gov**

3. Package specimens according to shipping requirements (refer to shipping requirement section).
4. Refer specimens to the UDOH Laboratory for confirmation.
5. Preserve original specimen pursuant to a criminal investigation.

REFERENCE

1. CDC and ASM. Basic Protocols for Level A Laboratories. www.bt.cdc.gov, www.asmusa.org


Francisella tularensis: Level A Laboratory Flowchart
Morphology: Aerobic, pleomorphic, minute (0.2 to 0.5 by 0.7 to 1.0 um), faintly staining, gram-negative coccobacilli.
Growth: Scant to no growth on sheep blood agar after >48 hours. Produces 1 to 2 mm gray to grayish white colonies on chocolate agar after >48 hours.
Peform all additional work in biosafety cabinet
Oxidase: Negative
Catalase: Weak positive
Beta-lactamase: Positive
Urease: Negative
Warning: Automated identification systems may key out as non-*F. tularensis* (e.g., *Haemophilius influenzae* and *Actinobacillus spp*.)
No (features not present)
Yes (features present)
Report: *F. tularensis* is ruled out; Continue identification per laboratory procedures
Report: Suspect, could not rule out; Refer to state public health laboratory for confirmation

GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *FRANCISELLA TULARENSIS*

PURPOSE

The procedures described below function to rule out or presumptively identify *F. tularensis* in clinical specimens or isolates.

SAFETY

All procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL 2) practices and the culture transferred to a certified Class II biological safety cabinet (BSC) as soon as *F. tularensis* is suspected. ***Francisella* is one of the most commonly reported bacterial infections acquired in the laboratory. Due to the highly infectious nature of this organism and its visual similarity to *Haemophilus influenzae,* the technique of smelling the culture plate SHOULD NOT be used!** Laboratory coats and gloves must be worn when processing specimens and performing tests. Safety glasses or eye shields are recommended.

ACCEPTABLE SPECIMENS: *Environmental/nonclinical samples and samples from announced events are not processed by Sentinel laboratories; please contact local law enforcement.*

Specimens of choice will be determined by the clinical presentation.

A. Blood Culture (Septicemic): Collect appropriate blood volume and number of sets per established laboratory protocols. Standard blood culturing system (10-20 ml/bottle). Transport directly to Sentinel Laboratory at room temperature. Hold at room temperature until placed onto the blood culture instrument or incubator. Do not refrigerate. Follow established laboratory protocol for processing blood cultures.

B. Biopsied tissue or scraping/aspirate of ulcer or lesion: a swab of the ulcer is an acceptable alternative. Submit tissue, scraping, or aspirate in a sterile container. For small tissue samples, add several drops of sterile normal saline to keep the tissue moist. Transport at room temperature for immediate processing. If processing of specimen is delayed, keep specimen chilled (2-8˚C).

C. Swabs: Obtain a firm sample of the advancing margin of the lesion. If using a swab transport carrier, the swab should be reinserted into the transport package and the swab fabric moistened with the transport medium inside the packet. Transport at 2-8˚C; room temperature is acceptable. If processing of specimen is delayed, keep specimen chilled (2-8˚C).

D. Lower respiratory tract (pneumonic) – sputum or aspirate

Transport specimen (>1 ml) in a sterile, screw-capped container at room temperature if transport will be < 2 hours. If transport will be 24 hours or less, store and transport at 4˚C.

E. Serum – for serological diagnosis

An acute phase specimen should be collected as soon as possible after onset of disease. A convalescent phase specimen should be collected 21 days after the acute specimen. Collect blood (a minimum of 5 ml) by venipuncture into a tube without anticoagulant. Allow blood to clot, separate serum into a separate tube. Refrigerate and transport as soon as possible.

MATERIALS

A. Media

1. General nutrient-rich agar: Sheep blood agar (SBA) or equivalent
2. Cysteine-supplemented agar: Chocolate agar (CA), Thayer-Martin (TM) agar, buffered charcoal yeast extract (BCYE), or other similar agar.
3. Selective agar: MacConkey agar (MAC)
4. Thioglycollate broth
5. Blood culture, standard blood culture system

B. Reagents

1. Catalase reagent (3% hydrogen peroxide)
2. Gram stain reagents
3. Oxidase reagent
4. Urease test

C. Equipment/Supplies

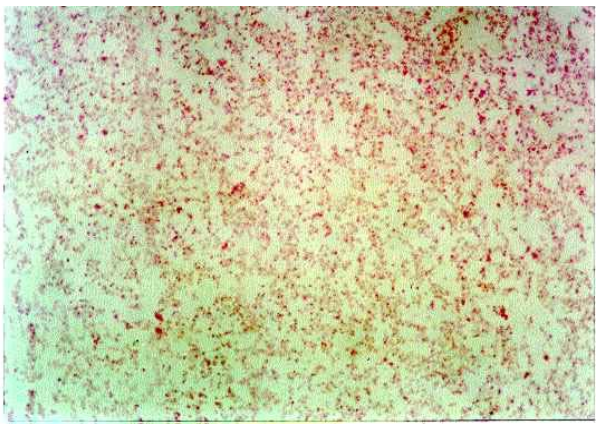
1. Microscope slides
2. Heat source for fixing slides
3. Staining rack for slides
4. Microscope with high power and oil immersion objectives
5. Bacteriologic loops, sterile
6. Incubator: 35-37˚C, ambient atmosphere, CO_2 acceptable

SPECIMEN PROCESSING & PRESUMPTIVE IDENTIFICATION

A. Gram stain

1. **Procedure**: Perform Gram stain procedure/quality control per standard laboratory protocol.
2. **Presumptive ID**
   a. Staining of *F. tularensis* often reveals the presence of tiny, 0.2 to 0.5 μm X 0.7 to 1.0 μm, pleomorphic, poorly staining, gram-negative coccobacilli seen mostly as single cells. The Gram stain interpretation may be difficult because the cells are minute and faintly staining. *F. tularensis* cells are smaller than *Haemophilus influenzae*. Bipolar staining is not a distinctive feature of *F. tularensis* cells.

Gram stain of *F. tularensis* (poorly staining)



B. Cultures

1. **Inoculation & Plating Procedure**: Use established inoculation and plating procedures using the media mentioned above. For tissues, use established laboratory procedure to inoculate media (e.g., grind, touch-preparation, or a sterile wood stick). Tape plates shut in 2 places to prevent inadvertent opening.
2. **Incubation**
   a. Temperature: 35-37˚C
   b. Atmosphere: Ambient, use of 5% CO_2 is acceptable
   c. Length of incubation: Hold primary plates for 5 days. If it is known that patient has been treated with bacteriostatic antibiotics, then hold plates for up to 7 days to allow bacteria recovery time.
3. **Colony characteristics of *F. tularensis***
   a. *F. tularensis* is a fastidious organism and grow better and faster if the media is supplemented with cysteine. The organism may initially be recovered on SBA, but upon subsequent passage will fail to grow on standard SBA. It does not grow well in liquid medium and broth should only be used if a compromised specimen is received. The broth will then need to be held for at least 10 days. Caution should be taken when handling and/or manipulating broth cultures to prevent aerosolization.
   b. On cysteine supplemented agar plates, it is a gray-white, opaque colony, usually too small to be seen at 24 hours. After incubation for 48 hours or more, colonies are about 1-2 mm in diameter, white to gray to bluish-gray, opaque, flat, with an entire edge, smooth and have a shiny surface.
   c. *F. tularensis* will not grow on MAC.

F. tularensis chocolate agar 72 hours

C. Biochemical reactions/tests

1. Oxidase test

1. Principle: Used to detect the presence of Oxidase enzymes associated with the Cytochrome respiratory system. The reagent is a dye that changes color in the presence of oxidase enzymes.
2. Specimen: Performed on samples of actively growing colonies from SBA or equivalent plates.
3. Reagents & Materials
   a. Oxidase reagent
   b. Whatman #1 filter paper or equivalent
   c. Disposable plastic loop
4. Procedure (can be performed using other validated laboratory procedure)
   a. Place 1 to 2 drops of Oxidase reagent on a piece of filter paper.
   b. Using a disposable plastic loop, mix a loopful of organisms from a plated culture into the reagent on the paper.
   c. Observe for the development of a light - dark blue color within 10 sec. of inoculation.
5. Characteristics
   a. Positive result: Development of a blue color within 10 sec. of inoculation.
   b. Negative result: No development of blue color.
   c. Some metals in bacteriological loops produce false positive reactions.

2. Urease test (Christensen's Method)

1. Principle: Used to determine an organism's ability to hydrolyze urea, forming ammonia by action of the enzyme urease.
2. Specimen: Actively growing culture of the test organism from non-selective media.
3. Reagents & Materials
   a. Urea agar
   b. Timer
   c. Sterile inoculation loop
4. Procedure
   a. Allow the urea agar to equilibrate to room temperature.
   b. Using a sterile inoculating loop, transfer a loopful of test organism to the surface of the urea agar slant.
   c. Incubate at 35-37˚C in ambient atmosphere.
   d. Set timer for 15 minutes.
   e. After 15 minutes of incubation, observe the inoculated slant for a color change to pink in the inoculated area. If no color change is observed, replace the slant in the incubator and observe it again after 24 hours of incubation.
5. Characteristics
   a. Positive result: Development of a pronounced pink color in the agar.
   b. Negative result: Absence of pink color.

3. Catalase test

1. Principle: Used to determine an organism's ability to breakdown hydrogen peroxide into Oxygen and water by action of the enzyme catalase.
2. Specimen: Actively growing culture of the test organism from non-selective media.
3. Reagents & Materials
   a. 3% Hydrogen Peroxide
   b. Glass microscope slide
   c. Sterile wooden applicator stick or loop
4. Procedure
   a. With the loop or applicator stick, transfer a small amount of growth from the agar plate onto the surface of a clean, dry glass slide.
   b. Place a drop of 3% hydrogen peroxide onto the growth on the slide.
   c. Observe for the evolution of bubbles of gas.
5. Characteristics
   a. Positive result: Production of gas bubbles
   b. Negative result: No gas bubbles produced

Additional Notes: Commercial biochemical identification systems are NOT recommended at this stage!

MAJOR CHARACTERISTICS: ***Francisella tularensis is suspected if the following conditions are met*:**

1. Tiny, poorly staining, Gram negative bacilli seen mostly as single cells (Gram stain morphology may be indistinct because the cells are so small)
2. Pinpoint colonies on chocolate and maybe on SBA at 48 hrs
3. No growth on MAC
4. Oxidase negative
5. Catalase weak positive or negative
6. Urease test negative

LIMITATIONS

A. The identification of *F. tularensis* should not be attempted with commercial identification systems because of the potential of generating aerosols and the high probability of misidentification.

B. Wild-type *F. tularensis* will grow initially on SBA but will grow poorly or not at all upon subsequent passages. Cysteine-enriched media (CA, TM, BCYE) would support growth of subcultures.

C. The most common misidentification of *F. tularensis* is *Haemophilus influenzae* and *Actinobacillus* species. Identification of isolates by using commercial identification systems is not recommended due to the high probability of misidentification. The Vitek NHI panel may give as high as 99% confidence to the identification of *Actinobacillus actinomycetemcomitans* with strains of *F. tularensis.*

D. Due to the highly infectious nature of this organism, susceptibility testing should not be attempted. Antibiotic therapy is streptomycin or gentamicin, with doxycycline or chloramphenicol as acceptable alternatives.

ACTIONS IF *FRANCISELLA TULARENSIS* IS SUSPECTED OR ISOLATED

- Correlate laboratory results with clinical findings.
- **As soon as possible contact the UDOH Laboratory and/or Epidemiology, the specimens should be split with one set forwarded to the UDOH for rapid/advanced testing.**

If *F. tularensis* is used as a bioterrorism agent in a covert or "unannounced" event, immediate and appropriate notification will help save others in the community.

UDOH CONTACT INFORMATION

- **Emergency #: Pager: 888-EPI-UTAH**
- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**
- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: **rrolfs@utah.gov**

1. Package specimens and/or isolate according to shipping requirements (refer to shipping requirement section).
2. Refer original specimen and/or isolate to the UDOH Laboratory (Level B/C Laboratory) for confirmation.
3. Preserve original specimen pursuant to a potential criminal investigation.

REFERENCES

1. CDC and ASM. Basic Protocols for Level A Laboratories. www.bt.cdc.gov, www.asmusa.org

2. Balows et al. 1988. Laboratory Diagnosis of Infectious Diseases. Springer-Verlag. New York, NY
3. Eitzen et al. 1998. Medical Management of Biological Casualities – Handbook. U.S. Army Medical Research. Fort Detrick, Frederick, MA
4. Murray et al. 1999. Manual of Clinical Microbiology 7th Edition. ASM Press. Washington, D.C.
5. NCCLS documents M2-A6, M100-S8
6. Weyant et al. 1999. Laboratory Protocols for Bioterrorism Response Laboratories For the Identification of *F. tularensis*. APHL. www.aphlnet.org
7. Weyant et al. 1995. Identification of Unusual Pathogenic Gram-Negative Aerobic and Facultatively Anaerobic Bacteria. Williams & Wilkens. Baltimore, MA

GUIDELINES FOR SENTINEL LABORATORIES FOR THE SAFE HANDLING OF VARIOLA VIRUS – SMALLPOX AND RELATED POX VIRUSES

PURPOSE

The information given below serves to explain how to safely handle specimens collected for smallpox identification.

SAFETY

Smallpox virus is highly infectious. Manipulation of specimen material should be avoided. If manipulation is necessary, it should be done by a vaccinated individual using Biosafety Level 3 practices.

- If smallpox is suspected, notify the UDOH Laboratory immediately for evaluation and referral.
- Smallpox is a medical and public health emergency even when criminal activity is not suspected. Diagnosis is made by clinical findings and confirmed by advanced techniques in Level D facilities.
- Sentinel laboratories should not attempt to culture or perform any diagnostic assays on specimens suspected of containing smallpox virus.
- Responsibility of the Sentinel Laboratory is limited to advising medical staff on specimen selection and with packing, shipping and communication of specimens from suspected case.

TESTS AVAILABLE AT UDOH LABORATORY

The following are rule out tests that are currently available at the UDOH Laboratory.

1. **Vaccinia virus**
2. **Varicella zoster virus (Chickenpox)**
3. **Variola major virus**
4. **Non-variola Orthopox viruses (Monkeypox)**

ACCEPTABLE SPECIMENS (for Variola, Vaccinia, Varicella and Non-variola Orthopox)

A. Biopsy: Aseptically place two to four portions of tissue into a sterile, leakproof, freezable container. If transport time will be ≤6 hours, transport at 4°C. Store specimens at -20°C to -70°C.

B. Scabs: Aseptically place scrapings/material into a sterile, leakproof, freezable container. If transport time will be ≤6 hours, transport at 4°C. Store specimens at -20°C to -70°C.

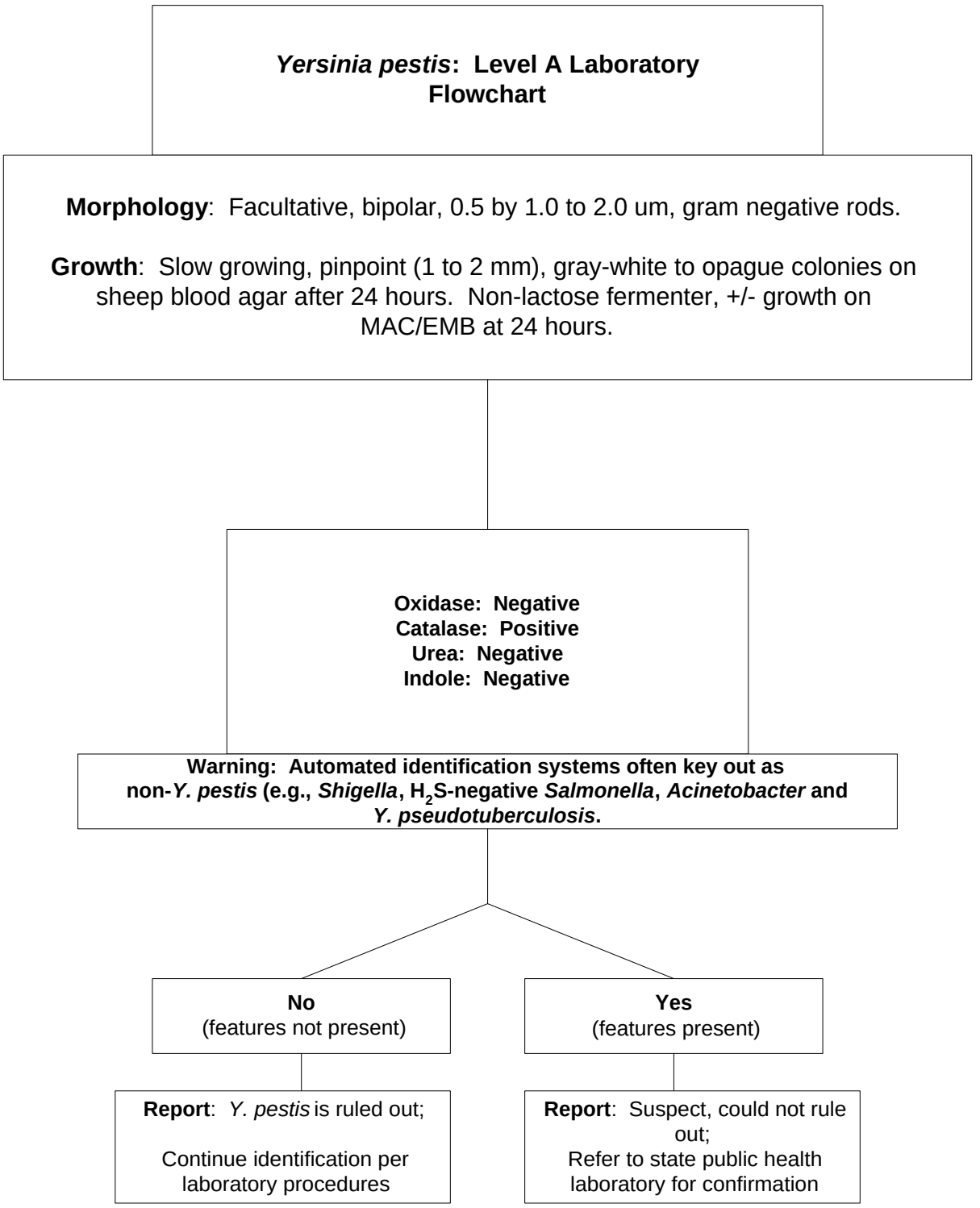
C. Vesicular fluid: Collect fluid from separate lesions onto separate sterile swabs. Be sure to include cellular materials from the base of each respective vesicle. If transport time will be ≤6 hours, transport at 4°C. Store specimens at -20°C to -70°C.

UDOH Contact Information

- **Emergency #: Pager: 888-EPI-UTAH**
- **Laboratory: 801-584-8400, Fax: 801-584-8486**
  Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
  Kim Christensen: 801-584-8449, email: kchriste@utah.gov
  Lori Smith: 801-584-8447, email: lhsmith@utah.gov
- **Acting Division Director: Teresa Garrett: 801-584-8400**
  email: teresagarrett@utah.gov
- **Bureau Director: Barbara Jepson: 801-584-8595, email: bjepson@utah.gov**
- Epidemiology: 801-538-6191, Fax: 801-538-9923
  Bob Rolfs: email: rrolfs@utah.gov

REFERENCE

1. Bioterrorism Response Guide for Clinical Laboratories, CDC. www.bt.cdc.gov
2. Utah Department of Health Smallpox Plan. www.


Yersinia pestis: Level A Laboratory Flowchart
Morphology: Facultative, bipolar, 0.5 by 1.0 to 2.0 um, gram negative rods.
Growth: Slow growing, pinpoint (1 to 2 mm), gray-white to opague colonies on sheep blood agar after 24 hours. Non-lactose fermenter, +/- growth on MAC/EMB at 24 hours.
Oxidase: Negative
Catalase: Positive
Urea: Negative
Indole: Negative
Warning: Automated identification systems often key out as non-*Y. pestis* (e.g., *Shigella*, H_2S-negative *Salmonella*, *Acinetobacter* and *Y. pseudotuberculosis*.
No (features not present)
Yes (features present)
Report: *Y. pestis* is ruled out; Continue identification per laboratory procedures
Report: Suspect, could not rule out; Refer to state public health laboratory for confirmation

GUIDELINES FOR SENTINEL LABORATORIES FOR THE PRESUMPTIVE IDENTIFICATION OF *YERSINIA PESTIS*

PURPOSE

The procedures described below function to rule out or presumptively identify *Y. pestis* in clinical specimens or isolates.

SAFETY

These procedures should be performed in microbiology laboratories that utilize Biological Safety Level 2 (BSL 2) practices. Laboratory coats and gloves shall be worn when processing specimens and performing tests. Safety glasses or eye shields are recommended. For safety considerations, all open manipulations or any activity that creates aerosols should be performed in a certified Class II biological safety cabinet (BSC).

ACCEPTABLE SPECIMENS - *Environmental/nonclinical samples and samples from announced events are not processed by Sentinel laboratories; please contact local law enforcement.*

Specimens of choice will be determined by the clinical presentation.

A. **Lower respiratory tract (pneumonic)**: Bronchial wash or transtracheal aspirate (≥1 ml). Sputum may be examined but it is not advised because of contamination by normal throat flora. Transport specimens in sterile, screw-capped containers at room temperature to the Sentinel Laboratory. If it is known that material will be transported from 2-24 hours after collection, then store the container and transport at 2-8˚C.
B. **Blood (septicemic)**: Collect appropriate blood volume and number of sets per established lab protocol. Note: In suspected cases of plague, an additional blood or broth culture (general nutrient broth) should be incubated at room temperature (22-28˚C), the temperature at which *Y. pestis* grows faster. Do not shake or rock the additional broth culture so that the characteristic growth formation of *Y. pestis* can be clearly visualized. Transport samples directly to the Sentinel Laboratory at ambient temperature. Hold them at ambient temperature until they are place onto the blood culture instrument or incubator. Do not refrigerate. Follow established laboratory protocol for processing blood cultures.
C. **Aspirate of involved tissue (bubonic) or biopsied specimen**: Liver, spleen, bone marrow, lung. Note: Aspirates may yield little material; therefore, a sterile saline flush may be needed to obtain an adequate amount of specimen. Syringe and needle of aspirated sample should be capped, secured by tape and sent to the Sentinel Laboratory. Submit tissue or aspirate in a sterile container. For small samples, add 1-2 drops of sterile normal saline to keep the tissue moist. Transport the sample at room temperature for immediate processing. Keep the specimen chilled if processing of the specimen will be delayed.
D. **Swabs**: A swab of tissue is not recommended. However, if a swab specimen is taken, the swab should be reinserted into the transport package for transport.

MATERIALS

A. Media

1. General nutrient-rich media: Sheep blood agar (SBA) and equivalent
2. General nutrient-rich broth: Brain heart infusion (BHI) and equivalent
3. Selective agar: MacConkey (MAC)
4. Blood culture, standard blood culture system

B. Reagents

1. Gram stain reagents
2. Wayson stain and/or Wright-Giemsa stain
3. Oxidase reagent
4. Catalase reagent (3% hydrogen peroxide)
5. Urease test (Christensen's)

C. Equipment/Supplies

1. Microscope slides
2. Heat source for fixing slides
3. Staining rack for slides
4. Microscope with high power and oil immersion objectives
5. Bacteriologic loops, sterile
6. Incubator: Ambient atmosphere, 28˚C and 35-37˚C

SPECIMEN PROCESSING & PRESUMPTIVE IDENTIFICATION

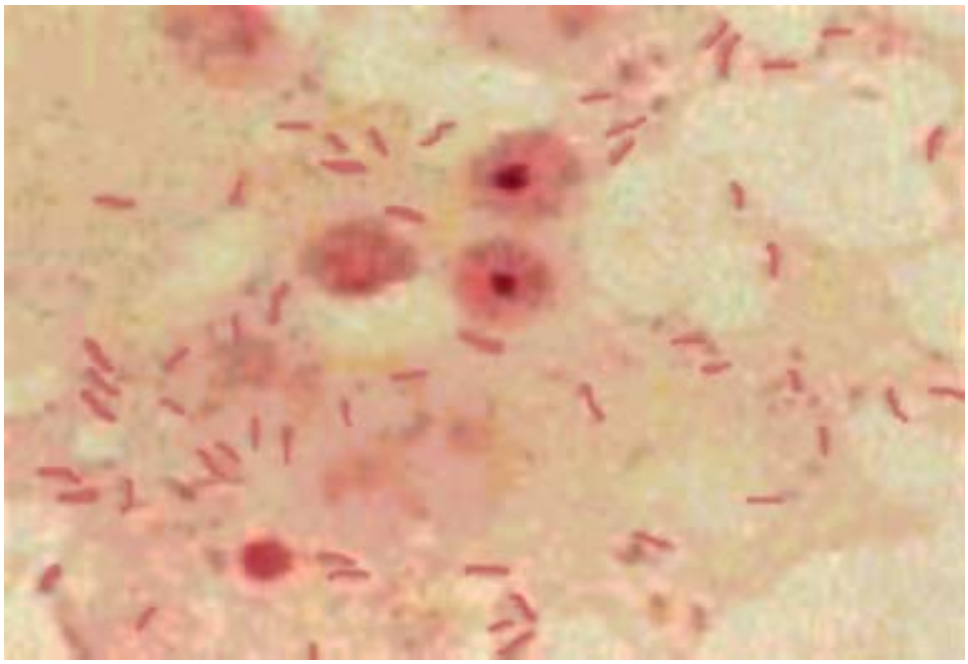
A. Stains and Smears

1. Gram stain

- **a. Procedure**: Perform Gram stain procedure/quality control per standard laboratory Protocol. Smears for staining may be prepared in order of likely positive results (i.e., cultures, bubo aspirates, tissue, blood, and sputum specimens).
- **b. Interpretation**: Direct microscopic examination of specimens and cultures by Gram stain can provide a rapid presumptive identification. Stained specimens containing *Y. pestis* often reveal plump, gram-negative rods (resembling safety pins), 1-2 μm by 0.5 μm, that are seen mostly as single cells or pairs and short chains in liquid media.
  Note: Patients with pneumonic plague may be secondarily infected with *Streptococcus pneumoniae*. Both of these organisms may be visualized in the sputum smears. It is imperative to evaluate such smears for the presence of gram-negative rods around the leukocytes (not necessarily intracellularly).

2. Other stains

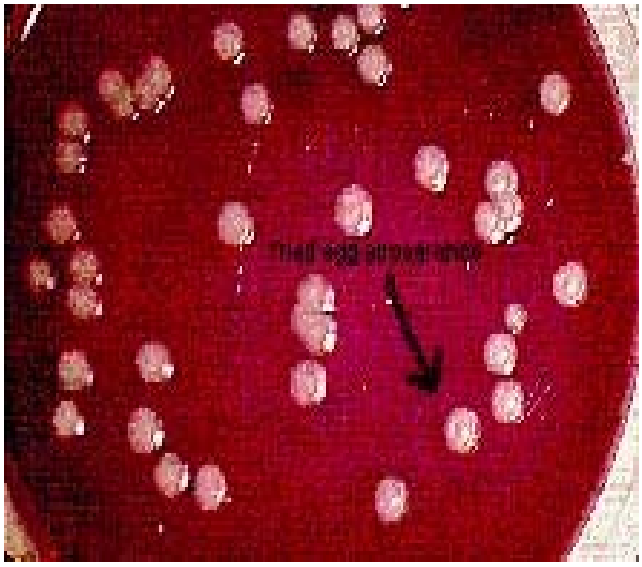
- **a. Wright-Giemsa stain**: Presence of bipolar cells in these smears should trigger the the suspicion of plague. The Wright stain often reveals the bipolar staining characteristics of *Y. pestis*, whereas the Gram stain may not. The Wright-Giemsa stains are the most reliable for accurately highlighting the bipolar staining.
- **b. Wayson stain**: Another polychromatic stain, can be used instead of Wright-Giemsa



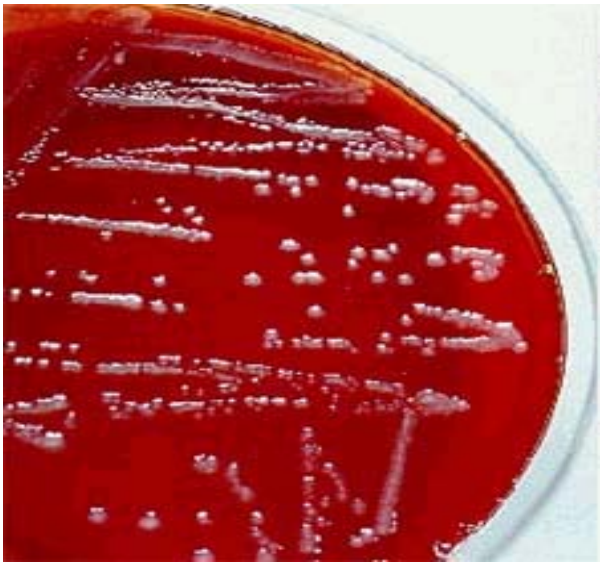
Y. pestis gram stain

B. Cultures

1. **Procedure:** Use established inoculation and plating procedures using the above mentioned media. For tissues, use established laboratory procedure to inoculate media (e.g., grind, touch-preparation, or by using a sterile wood stick). Then, tape plates shut in 2 places to prevent inadvertent opening.
2. **Incubation of cultures**
   a. Temperature: 28˚C (optimal); 35-37˚C (grow more slowly)
   b. Atmosphere: Ambient, use of 5% CO_2 is acceptable
   c. Length of incubation: Hold primary plates for 5 days. Plates should be held for up to 7 days if the patient has been treated with antibiotics.
3. **Colony characteristics of *Y. pestis***
   a. Agar plates: *Y. pestis* grows as gray-white, translucent colonies, usually too small to be seen as individual colonies after 24 hours. After incubation for 48 hours, colonies are about 1-2 mm in diameter, gray-white to slightly yellow and opaque. Under 4X enlargement, after 48-72 hours of incubation, colonies have a raised, irregular "fried-egg" appearance, which becomes more prominent as the culture ages. Colonies also can be described as having a "hammered copper" shiny surface. There is little or no hemolysis of the sheep red blood cells. *Y. pestis* will grow as small, non-lactose fermenting colonies on MAC agar.

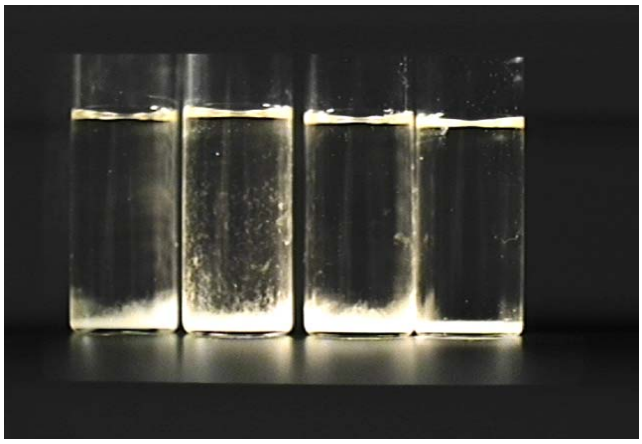


Characteristic "fried egg" morphology



Characteristic "hammered copper" morphology

b. Broth tubes: *Y. pestis* grows in clumps that are typically described as "flocculent" or "stalactite" in appearance when the broth culture is not shaken or mixed. At 24 hours the growth is seen as clumps that hang along the side of the tube. After 24 hours, the growth settles to the bottom of the tube described as "cotton fluff".



Note: *Yersinia pestis* organisms are not fastidious and grow well on any nutrient medium, although at 37°C the growth will be slower and the colonies smaller. *Y. pestis* grows faster at room temperature, however, the diagnostic fraction (F1 antigen) is expressed only at temperatures greater than 28°C. When plague is suspected, duplicate cultures should be set up at 37°C and 28°C.

C. Biochemical reactions/tests

1. Oxidase

1. **Principle:** Used to detect the presence of Oxidase enzymes associated with the Cytochrome respiratory system. The reagent is a dye that changes color in the presence of oxidase enzymes.
2. **Specimen:** Performed on samples of actively growing colonies from SBA or equivalent plates.
3. **Reagents & Materials**
   a. Oxidase reagent
   b. Whatman #1 filter paper or equivalent
   c. Disposable plastic loop
4. **Procedure** (can be performed using other validated laboratory procedure)
   a. Place 1 to 2 drops of Oxidase reagent on a piece of filter paper.
   b. Using a disposable plastic loop, mix a loopful of organisms from a plated culture into the reagent on the paper.
   c. Observe for the development of a light-dark blue color within 10 seconds of inoculation.
5. **Characteristics**
   a. Positive result: Development of a blue color within 10 sec. of inoculation.
   b. Negative result: No development of blue color.
   c. Some metals in bacteriological loops produce false positive reactions.

2. Urease test (Christensen's Method)

1. **Principle:** Used to determine an organism's ability to hydrolyze urea, forming ammonia by action of the enzyme urease.
2. **Specimen:** Actively growing culture of the test organism from non-selective media.
3. **Reagents & Materials**
   a. Urea agar
   b. Timer
   c. Sterile inoculation loop
4. **Procedure**
   a. Allow the urea agar to equilibrate to room temperature.
   b. Using a sterile inoculating loop, transfer a loopful of test organism to the surface of the urea agar slant.
   c. Incubate at 35-37°C in ambient atmosphere.
   d. Set timer for 15 minutes.
   e. After 15 minutes of incubation, observe the inoculated slant for a color change to pink in the inoculated area. If no color change is observed, replace the slant in the incubator and observe it again after 24 hours of incubation.
5. **Characteristics**
   a. Positive result: Development of a pronounced pink color in the agar.
   b. Negative result: Absence of pink color.

3. **Catalase test**
    1. **Principle:** Used to determine an organism's ability to breakdown hydrogen peroxide into oxygen and water by action of the enzyme catalase.
    2. **Specimen:** Actively growing culture of the test organism from non-selective media.
    3. **Reagents & Materials**
        a. 3% Hydrogen peroxide
        b. Glass microscope slide
        c. Sterile wooden applicator stick or loop
    4. **Procedure**
        a. With the loop or applicator stick, transfer a small amount of growth from the agar plate onto the surface of a clean, dry glass slide.
        b. Place a drop of 3% hydrogen peroxide onto the growth on the slide.
        c. Observe for the evolution of bubbles of gas.
    5. **Characteristics**
        a. Positive result: Production of gas bubbles
        b. Negative result: No gas bubbles produced

Additional Notes: Commercial biochemical identification systems are NOT recommended at this stage!

MAJOR CHARACTERISTICS: ***Y. pestis is suspected if the following conditions are met:***

a. Bipolar staining rod (Wright-Giemsa) on direct smear
b. Pinpoint colony at 24 hour on sheep blood agar
c. Non-lactose fermenter, may not be visible on MacConkey/EMB at 24 h
d. Non-motile. *Y. pestis* is the only non-motile species of the *Yersiniae*
e. Oxidase and urease negative
f. Catalase positive
g. Growth often better at room temperature (22-28^{o}C).

Any isolate containing the major characteristics noted above should be suspected as *Y. pestis*. A bacterial isolate from the respiratory tract, blood or lymph node, that is identified as *Y. pestis* or any Yersinia species by commercial identification products (including rapid methods) should be forwarded to the UDOH Laboratory for confirmation**.**

LIMITATIONS

A. *Y. pestis* will grow on general nutrient-rich media, but its growth rate is slower than that of most other bacteria; therefore, its presence may be masked by organisms that replicate faster.
B. Bipolar staining of cells is not an exclusive feature limited to *Y. pestis*. *Yersinia* spp., enteric bacteria, and other gram-negative organisms, particularly *Pasteurella* spp. can exhibit the same growth feature.
C. Characteristic clumped growth in unshaken broth culture is not an exclusive feature of *Y. pestis*. Some *Y. pseudotuberculosis* and *Streptococcus pneumoniae* can exhibit

the same growth feature.

D. Some of the automated identification systems do not identify *Y. pestis* adequately. *Y. pestis* has been falsely identified as *Y. pseudotuberculosis, Shigella,* H_2S-negative *Salmonella,* or *Acinetobacter*. *Y. pestis* is alkaline slant/acid butt in triple sugar iron. In most conventional biochemical or commercial identification systems, the organism appears relatively inert, making further biochemical testing of little value.

ACTIONS IF SUSPECTED *Y. PESTIS* IS ISOLATED

- Correlate laboratory results with clinical findings.
- **As soon as possible contact the UDOH Laboratory and/or Epidemiology, the specimens should be split with one set forwarded to the UDOH for rapid/advanced testing.** If *Y. pestis* is used as a bioterrorism agent in a covert or "unannounced" event, immediate and appropriate notification will help save others in the community.

    - Emergency #: Pager: 888-EPI-UTAH
    - **Laboratory: 801-584-8400, Fax: 801-584-8486**
      Microbiology: **Jana Coombs: 801-584-8449, email: jcoombs@utah.gov**
      Kim Christensen: 801-584-8449, email: kchriste@utah.gov
      Lori Smith: 801-584-8447, email: lhsmith@utah.gov
    - **Acting Division Director: Teresa Garrett: 801-584-8400**
      email: teresagarrett@utah.gov
    - **Bureau Director: Barbara Jepson: 801-584-8595, email:bjepson@utah.gov**
    - Epidemiology: 801-538-6191, Fax: 801-538-9923
      Bob Rolfs: email: **rrolfs@utah.gov**

- Package specimens and/or isolate according to shipping requirements (refer to shipping requirement section).
- As soon as possible, refer original specimen and/or isolate to the UDOH Laboratory for confirmation.
- Preserve original specimen pursuant to a potential criminal investigation.

REFERENCES

1. Balows et al. 1988. Laboratory Diagnosis of Infectious Diseases. Springer-Verlag. New York, NY
2. CDC and ASM. Basic Protocols for Level A Laboratories. www.asmusa.org, www.bt.cdc.gov.
3. Eitzen et al. 1998. Medical Management of Biological Casualties—Handbook . U.S. Army Medical Research. Fort Detrick, Frederick, MA
4. Koneman EW, SD Allen, WM Janda, PC Schreckenberger, and WC Winn Jr. (eds.). 1997 Enterobacteriaceae. In, *Color Atlas and Textbook of Diagnostic Microbiology*, 5th ed. pp. 171-252. Lippincott, Philadelphia, PA
5. Murray et al. 1999. Manual of Clinical Microbiology 7th Edition. ASM Press. Washington, D.C.
6. NCCLS documents M2-A6, M100-S8
7. Perry RD and JD Fetherston. 1997. *Yersinia pestis* – Etiologic agent of plague. Clin Microbiol Rev 10:35-66.
8. Weyant et al. 1999. Laboratory Protocols for Bioterrorism Response Laboratories For the Identification of *Yersinia pestis.* APHL. www.aphlnet.org

Guidelines For the Safe Transport of Infectious Substances

I. Introduction

Packaging and shipping regulations are provided to protect postal, airline and other transport industry personnel from exposure to microorganisms that escape from broken, leaking or improperly packaged material. These regulations have been put together by several agencies within the federal government and by private industry associations. The regulations vary somewhat, but all of them give the responsibility to the **shipper** to correctly **classify, package, label, and prepare documentation** for all shipments of diagnostic and infectious material. All national and international regulations require that anyone handling, packaging or shipping infectious substances must be a trained person.
(See **Appendix A** for regulation sources)

II. Classification (From the current United Nations (UN) Recommendations on the Transport Of Dangerous Goods.)

A Diagnostic Specimen:
Diagnostic specimens are not considered to be hazardous material because they pose a negligible threat to the public health. They should have a relatively low probability of containing pathogens, and are being shipped for the purpose of testing other than for presence of pathogens.

B Infectious Substance:
Infectious substances are classified as **hazardous material** that requires specific packaging. These samples are defined as infectious substances:

1. All **cultures** containing or suspected of containing an agent that may cause infection; (see infectious agent list, **Appendix B.**)
2. Human or animal samples likely to contain an infectious agent.

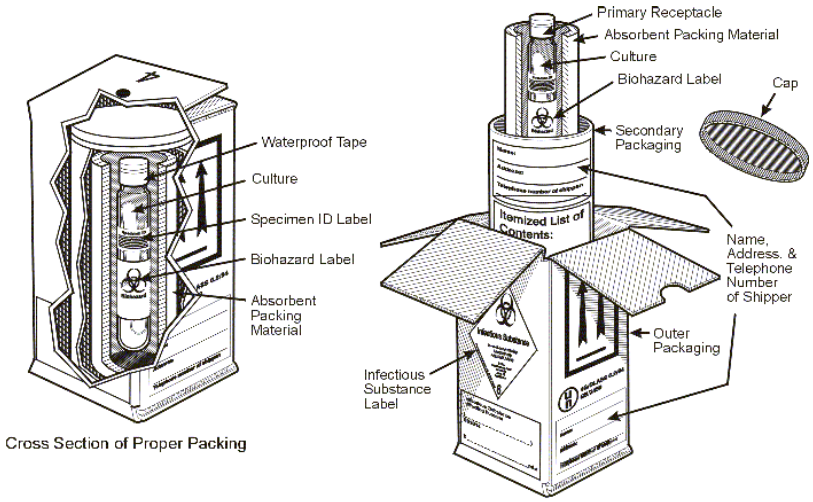
III. Shipment of Infectious Substances by Commercial Carrier

A Planning

1. Call the recipient to verify the shipping address and obtain the name and phone number of the contact person. Find out when the contact person will be able to receive the shipment.
2. Shipments should not go out on Thursday or Friday unless the recipient guarantees that they will be there to receive the package on the weekend.

B Packaging

Proper packaging includes the classification, packing, labeling, and documentation of materials for shipment. Samples must be packaged to withstand leakage of contents, shocks, pressure changes and other conditions likely to occur during ordinary handling in transportation.

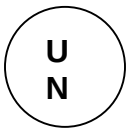


Packing and Labeling of Infectious Substances

1. The **primary sample container** has to be waterproof and leakproof.
    a. Seal culture plates and screw-capped tubes with tape or Parafilm.
    b. Wrap the specimen container with enough absorbent material to absorb the entire liquid contents in the event of leakage.
    c. Put the sample container in a zip-locked biohazard bag. Put solid culture containers in one zip-locked bag. Put liquid culture containers in **two** bags.
    d. Pre-freeze samples that will be shipped frozen.

2. The **secondary container** is part of a complete packaging system. It should be unbreakable, waterproof, leak proof and have a biohazard label on the outside. (See Appendix C for certified shipping systems, source information and packing instructions.)

3. A certified **outer shipping container** is strong enough to hold the capacity and mass indicated on the bottom of the box. Choose the appropriate package.
    a. It must meet the UN class 6.2 specifications and packaging instructions (PI) 602 and bear the UN Packaging Specification Marking. Containers and packaging systems must be 4G Class 6.2/98 or less than three years from the year of certification design.



4G CLASS 6.2 / 99
CAN / 8-2 SAF-T-PAK

    b. Each shipper comes with the required inner packaging and labels. Do not make any substitutions or the UN-certification becomes invalid. If the secondary container is re-usable, you may use a refurbishment kit for each shipper. Ensure there are no holes or dents and remove previous labels from recycled shippers.

    c. Follow the **closing instructions** included with each UN-certified packaging system.

    d. If over-packs are used, the shipping package and the over-packs must be marked and labeled identically. An additional label is required on the over-pack:

"Inner packages comply with
prescribed specifications"

C Labeling: Apply labels to a flat surface without overlap or corner wrap**.**

1. **Hazard Labels for Dangerous Goods** must be displayed on packages with infectious substances and/or dry ice.

a **Hazardous Class 6.2** Infectious Substances

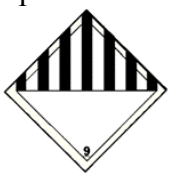
Printing on the label should state:

Etiologic agents
Biomedical material
In case of damage or leakage
Notify Director CDC, Atlanta, Georgia
(404) 633-5313

Apply the Class 6.2 label on the blank diamond marked on the outside of the outer package.

b **Miscellaneous Hazard Class 9** Dry Ice

The Hazard Class 9 dry ice label is only required for shipments containing dry ice. The weight of the dry ice in kg is hand-written on the white portion of the label.



Apply the Miscellaneous Hazard label on the side of the box opposite the Hazardous Substance label.

The UN shipping name label for dry ice should be in the format:

Carbon dioxide, solid
(Dry ice)
UN1845
_____ kg.

Place this label next to the Miscellaneous Hazard label

2. The **Orientation Labels** are printed on opposite sides of the shipping container not covered by the hazard warning labels. Do not cover them.

3. **A UN shipping name label** is required for each **infectious organism** in a shipment. The UN shipping label must have the exact format:

Infectious substances,
Affecting humans
(*Escherichia coli*)
UN2814
X_____ mL.

It must be affixed to the outside package next to Hazard Class 6.2 label.

4. Apply an **Address Label** on one side of the box with the following information:

a The receiver's name, shipping address (no P.O. Boxes) and telephone number, including the area code.

b The shipper's name, address and telephone number

c Temperature storage requirements of the package contents (optional)

D Documenting

Complete the **forms and letters** that will be enclosed with the sample.

a **Memo**: All shipments of infectious substances must include a **memo on letterhead** (There is an example in **Appendix D**) Insert one of these forms on top of the secondary container before closing the outer shipping package.

b **Test request**: If a sample is being forwarded from another lab, enclose a copy of the referral test request form.

2. **Shipping record file**: Make a copy of the lab forms and slips to keep in a shipping record file folder. Keep shipping record folders for two years.

3. Complete **shipping documents** provided by the commercial carrier**:**

a Commercial air shipments require an **Air Waybill** (tracking document). Include the name and telephone number of the person responsible for the shipment. This person must be knowledgeable and accessible 7 days a week, 24 hours a day. Apply the Waybill, in a shipping pouch with an address window, to the top surface of the closed package.

b Infectious substances require a **Dangerous Goods Declaration**. Follow exact directions to avoid a fine.

(1) Insert the Dangerous Goods Declaration forms into a shipping pouch and apply the pouch to the bottom of the package.

(2) The attachment pouches may overlap the edge of the top and bottom of the package. Make sure that the edges do not overlap any of the labels or markings on the side

E Shipping

Some commercial carriers will not ship infectious substances. Call your local carrier to make sure they will accept an infectious substance.

IV. Local Transport of Diagnostic and Infectious Samples

Local transport, usually performed by a courier service, may include the transfer of specimens from a doctor's office / hospital to a laboratory, or from one laboratory to another. Safe transport by this means is as important as for air shipment. The contents of a sample should not have any possibility of escaping from the package under normal conditions of transport.

A Packaging

1. The primary sample container has to be waterproof and leakproof.
    a Seal culture plates and screw-capped tubes with tape or Parafilm.
    b Wrap the specimen container with absorbent material to absorb any leakage and secure with tape.
2. Put the specimen container in a zip-locked biohazard bag with the laboratory name, address, and telephone number. Put containers with liquid culture media in **two** bags.

B Labeling

1. Attach a label with the name, address, and telephone number of the recipient and storage requirements.
2. Put the specimen identification form or test request form in the outside pocket of the specimen biohazard bag. **Do not** put the form **inside** the specimen bag.

C Transporting

1. The sample containers should be placed in a leakproof, unbreakable transport box with a secure, tight-fitting cover and a biohazard label. Frozen specimens should be put into a labeled, insulated box with dry ice.
2. The transport box should be carried to the courier vehicle and secured in position for transport.
3. The courier vehicle should carry a spill kit with absorbent material, disposable gloves, a chlorine disinfectant, and a leak-proof waste disposal container.

Appendix A. Regulation References For the Transportation of Infectious Substances

1. **Public Health Service 42 CFR Part 72. Interstate Transportation of Etiologic Agents.**
   http://www.cdc.gov/od/ohs/biosfty/shipregs.htm

2. **Department of Transportation. 49 DFR Parts 171-180. Hazardous Materials Regulations.** Applies to the shipment of both biological agents and clinical specimens.
   http://hazmat.dot.gov/rules.htm

3. **United States Postal Service. 39 CFR Part 111. Mail ability of Etiologic Agents.** From the Domestic Mail Manual 124.38.
   http://www.access.gpo.gov

4. **Occupational Health and Safety Administration (OSHA). 29 CFR Part 1910. 1030.** Provides minimal packaging and labeling requirements for transport of blood and body fluids within the laboratory and outside of it.
   http://www.osha.gov

5. **Dangerous Goods Regulations (DGR). International Air Transport Association (IATA).** These regulations followed by the airlines provide packaging and labeling requirements for infectious substances and materials, as well as for diagnostic specimens.
   http://www.iata.org/cargo/dg/

6. **Guidelines for the Safe Transport of Infectious Substances and Diagnostic Specimens.** World Health Organization, 1997.
   http://www.who.int/emc/biosafety.html

Appendix B. Infectious Agents Listed in 42 CFR Part 72
Interstate Shipment of Etiologic Agents

Bacterial Agents

Acinetobacter calcoaceticus
Actinobacillus – all species
Actinomycetaceae – all members
Aeromonas hydrophilia
Arachnia propionica
Arizona hinshawii – all serotypes
Bacillus anthracis
Bacteroides spp.
Bartonella – all species
Bordetella – all species
Borrelia recurrentis, B. vincenti
***Brucella* – all species**
Burkholderia mallei
Burkholderia pseudomallei
Campylobacter (Vibrio) foetus,
C. (Vibrio) jejuni
Chlamydia psittaci, C. trachomatis
Clostridium botulinum,
Cl. chauvoei
Cl. haemolyticum, Cl. histolyticum
Cl. novyi
Cl. septicum
Cl. tetani
Corynebacterium diphtheriae
C. equi
C. haemolyticum
C. pseudotuberculosis
C. pyrogenes
C. renale
Edwarsiella tarda
Erysipelothrix insidiosa
Escherichia coli – all enteropathogenic serotypes
Francisella [Pasteurella] tularensis
Haemophilus ducreyi
H. influenzae
Klebsiella – all species and all serotypes
Legionella – all species and all Legionella-like organisms
Leptospira interrogans – all serovars
Listeria – all species
Mimae polymorhpa
Moraxella – all species
Mycobacterium – all species
Mycoplasma – all species
Neisseria gonorrhoeae
N. meningitidis
Nocardia asteroides
Pasteurella – all species
Plesiomonas shigelloides
Proteus – all species
Salmonella – all species and all serotypes
Shigella – all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pneumoniae
Streptococcus pyrogenes
Treponema careteum
T. pallidum
T. pertenue
Vibrio cholerae
V. parahaemolyticus
Yersinia (Pasteurella) pestis
Y. enterocolitica

Fungal Agents

Blastomyces dermatitidis
Coccidioides immitis
Cryptococcus neoformans
Histoplasma capsulatum
Paracoccidioides brasiliensis

Appendix B. (cont')

Viral and Rickettsial Agents

Adenoviruses – human – all types
Arboviruses – all types
Coxiella burnetii
Coxsackie A and B viruses – all types
Creutzfeldt – Jacob agent
Cytomegaloviruses
Dengue viruses – all types
Ebola viruses
Echoviruses – all types
Encephalomyocarditis virus
Hemorrhagic fever agents including, *but not limited to, Crimean hemorrhagic fever (Congo) Junin, Machupo viruses, and Korean hemorrhagic fever viruses*
Hepatitis associated materials *(hepatitis A, hepatitis B, hepatitis noA-nonB)*
Herpesvirus – all members
Infectious bronchitis – like virus
Influenza viruses – all types
Kuru agent
Lassa virus
Lymphocytic choriomeningitis virus
Measles virus
Mumps virus
Parainfluenza viruses – all types
Polioviruses – all types
Rabies virus – all strains
Reoviruses – all types
Respiratory syncytial virus
Rhinoviruses – all types
Rickettsia – all species
Rocha limaea Quintana
Rotaviruses – all types
Rubella virus
Simian virus 40
Tick-borne encephalitis virus complex, including Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever, and Central European encephalitis viruses
Vaccinia virus
Varicella virus
Variola major and Variola minor viruses
Vesicular stomatis viruses – all types
White poxviruses
Yellowfever virus

Appendix C. UN-Certified Shipping Systems

For Hazard Class 6.2, Infectious Substances

With Closing Instructions

SAF-T-PAK, Inc.
101, 17872 – 106 Avenue
Edmonton, Alberta, Canada T5S 1V4
Phone: 800-841-7484
FAX: 403-486-0235Web:
http://www.saftpak.com

VWR Scientific Products
Sales: (800) 932-5000
Sales Fax: (800) 477-4897
Internet: http://www.vwrsp.com

Speci-FREEZ Insulated Shipper
VWR cat. No. 11217- 086 4/$56.00
Use the insulated shipper with the Infecon 3000 complete packaging system for transporting specimens that must be kept frozen or refrigerated. It is designed to hold the smaller package securely without shifting as the dry ice dissipates.

When packed with approx. 22 lbs. Dry ice, this lightweight, insulated shipper keeps contents frozen for 76 hours. Transporter has 1 ½ " thick expanded polystyrene sides and lid, enclosed in a 200 lb.-test fiberboard carton for protection. It contains all the necessary hazard labels, handling labels and instructions.

Infecon 3000 Infectious Substance Shipper
VWR cat. No. 11217-660 12/$219.34

Refurbishment Kit **VWR cat. No. 11217-114 4/$79.69** contains outer box, O-ring, coil, instructions, declaration and labels.

Appendix C. (Cont')

Use an Infecon 3000 Infectious Substance Shipper for **small** samples
(Less than 500 ml and less than 3" X 4") shipped at **ambient** temperature.
The complete, ready-to-use pack includes a pressure vessel, cushioning material, absorbent, labels, outer box, and instructions. The 1.25L polypropylene pressure vessel (secondary container) with O-ring is airtight and leak-proof, autoclavable and will withstand up to 95-kPa pressure. The sturdy outer box is imprinted with instructions and clearly displayed UN markings. The pack may be completely refurbished for economical re-use.

Closing instructions for the Infecon 3000

1. Use the entire system as directed. Do not alter the inner packing material. Failure to use the packaging system properly makes you liable for any damage or injury resulting from breakage or exposure to the infectious contents of the package.
2. Seal the primary container in a zip-locked biohazard bag with the lab's name, address and phone number.
3. Wrap the bagged container snugly in a piece of bubble wrap and secure it with pressure sensitive tape. Put an absorbent pad, sufficient to absorb any liquid, into the bottom of the wide-mouthed jar with the orange screw cap.
4. Place the wrapped primary container inside the jar and fill voids with bubble wrap.
5. If you are shipping more than one specimen in the same package, do not allow primary containers to come in contact with one another during shipment.
6. Make sure the O-ring seal is smooth and intact. Screw the cap on.
7. Tape an itemized list of contents on the outside of the jar.
8. Put the fiberboard coil into the outer shipping package.
9. Place the closed jar inside the fiberboard coil.
10. Leave the outer package open until the shipping documents are prepared.
11. Compare the information on the Air Waybill and Dangerous Goods Declaration to the information on the letterhead memo for accuracy.
12. Put the memo on top of the inner pack and close the box.
13. Tape the flaps, top and sides with 3" shipping tape.
14. Apply the Air Waybill, Dangerous Goods Declaration and hazard labels on the outside of the package according to the section on labeling and documentation.

Appendix D. Example of a Memo
To Accompany Infectious Substance Shipments

From: Name / Title
Facility Name
Address
Telephone: _____

To: Name / Title
Facility Name
Address
Telephone: _____

Date:

Re:

Explain what is being sent and why.

Storage conditions:

_____
(Signature)

Instructions: Supply the required information and print on letterhead paper.

Appendix E. Clinical Specimen Selection for Agents of Bioterrorism

Bacillus anthracis "Anthrax"

Cutaneous lesions - Vesicular Stage –
Collect fluid from intact vesicles on sterile swab(s) from previously unopened vesicles. The organism is best demonstrated in this stage.

Transport – Directly to lab at Room Temperature
For transport >1 h and <24 h, transport at 2-8°C
Storage - >1 hour at 2-8°C

Cutaneous lesions - Eschar Stage –
Collect eschar material by CAREFULLY lifting the eschar's outer edge; insert sterile swab, Then slowly rotate for 2-3 seconds beneath the edge of the eschar without removing it.

Transport – Directly to lab at Room Temperature
For transport >1 h, transport at 2-8°C
Storage - >1 hour at 2-8°C

Stool –
Collect 5-10 grams in a clean, sterile, leak-proof container. Cary-Blair or equivalent transport Media is acceptable.

Transport – Unpreserved stool - within 1 hour to laboratory
For transport >1 h, transport at 2-8°C
Storage – 2-8°C

Rectal swab –
For patients unable to pass a specimen, obtain a rectal swab by carefully inserting a swab 1-Inch beyond the anal sphincter.

Transport – Directly to laboratory at room temperature
For transport time >2 h, transport at 4°C
Storage – 2-8°C

Blood –
Collect per institution's procedure for routine blood cultures
A purple, blue or green-top blood tube may be requested for additional testing.

Transport – Directly to laboratory at room temperature
Storage - Depends on instrument

Sputum –
Collect >1 ml expectorated lower respiratory specimen into a sterile, leakproof container.

Transport – Directly to lab at Room Temperature
For transport >1 h and <24 h, transport at 2-8°C
Storage - >1 hour at 2-8°C

CSF, tissue, autopsy samples –
Collect aseptically and place in sterile container.

Transport – Directly to laboratory at room temperature.

Nasal swabs –
See page 20 of the Level A Manual for Agents of Bioterrorism for information on when and how to collect a nasal swab.

Specimen Selection for Agents of Bioterrorism

Brucella sp. "Brucellosis"

Serum –
For serologic diagnosis, an acute phase specimen should be collected as soon as possible after onset of disease. A convalescent phase specimen should be collected >14 days after the acute specimen. At least 1 ml is recommended.

Transport - ~2 hours at Room Temperature
For transport >2 hours, transport at 2-8°C
Storage – (-)20˚C

Blood or Bone Marrow –
These are the sources from which *Brucella* is most often isolated.
Collect per institution's procedure for routine blood cultures
A green, blue or purple-top blood tube may be requested for additional testing.

Transport - Room Temperature
Storage – Depends on instrument

Spleen, Liver or abscess –
Collect aseptically and place in sterile container. Tissue must be kept moist, add several Drops of sterile saline if necessary. DO NOT add formalin.

Transport – 2-8°C
Storage - 2-8°C

Burkholderia spp. "Glanders" and "Melioidiosis"

Blood –
Collect per institution's procedure for routine blood cultures.
A green, blue or purple-top blood tube may be requested for additional testing.

Transport - Room Temperature
Storage – Depends on instrument

Serum –
Use a red top or separator type tube to obtain serum. At least 1 ml of serum should be collected.

Transport – 2-8°C
Storage – 2-8°C; if a several day delay is anticipated, the serum may be frozen

Urine –
Collect a midstream clean-catch specimen or a catherization specimen.

Transport – 2-8°C
Storage – 2-8°C

***Burkholderia* spp. "Glanders" and "Melioidiosis"**

Abscesses, tissue aspirates, fluids –
Collect tissues and fluids rather than swabs, when possible. Collect aseptically and place in a Sterile container. Tissue must be kept moist, add several drops of sterile saline if necessary. DO NOT add formalin.

Transport – 2-8°C
Storage - 2-8°C

Special situations –
Throat, nasal, skin or sputum specimens may be helpful in screening exposed individuals if a release of *B. mallei* or *B. pseudomallei* has been confirmed. If this occurs, please contact the UDOH Laboratory for further instructions.

***Clostridium botulinum* "Botulism"**

Specimen(s) of choice for confirming botulism:

a. **Serum (not for infants)**
b. **Wound/tissue**
c. **Stool and incriminated food**

Stool –
Collect stool and place into a sterile, unbreakable container and label carefully. 10-50 grams (Walnut size) of stool is recommended. In infants, botulism has been confirmed with only "pea-sized" stool samples.

Transport – 2-8°C
Storage – 2-8°C; if a several day delay is anticipated, the stool may be frozen.

Enema fluid –
Purge with approximately 20 ml of sterile non-bacteriostatic water to minimize dilution of toxin. Place into a sterile, unbreakable, leakproof container and label carefully.

Transport -- 2-8°C
Storage -- 2-8°C

Gastric aspirate or vomitus –
Collect approximately 20 ml in a sterile, leakproof container.

Transport -- 2-8°C
Storage -- 2-8°C

Clostridium botulinum **"Botulism"**

Serum –

Use red top or separator type tubes to obtain serum (no anticoagulant). Samples should be collected as soon as possible after the onset of symptoms and before antitoxin is given. Approximately 20 ml of whole blood should be collected or 10 ml of serum. Serum volumes of less than 3 ml will provide inconclusive results. Serum should be obtained from whole blood prior to transport to the laboratory as it typically undergoes excessive hemolysis during transit. In infants, serum is generally not useful since the toxin is quickly absorbed before the serum can be obtained.

Transport – 2-8°C

Storage – 2-8°C; if a several day delay is anticipated, the serum may be frozen.

Tissue, wounds or exudates –

Collect aseptically and place into sterile container. Specimens should placed in Port-A-Cul Vials and sent to the laboratory.

Transport – Room Temperature

Storage – Room Temperature

Postmortem –

Obtain specimens of intestinal contents from different levels of the small and large intestines. Obtain gastric contents as appropriate. Place approximately 10 grams of specimen into a sterile, leakproof container.

Transport -- 2-8°C

Storage -- 2-8°C

Culture –

Ship suspicious isolates anaerobically (overlay liquid media with 2-inch layer of sterile petroleum jelly; melt/temper prior to overlaying culture).

Transport – Room Temperature or Refrigerated

Storage – Room Temperature or Refrigerated

Food Specimens –

Foods should be left in their original containers if possible, or placed in sterile, leakproof containers. Place containers individually in leakproof containers to prevent cross-contamination during shipment. Empty containers with remnants of suspected foods can be examined. Foods most likely to allow growth of *C. botulinum* will have a pH of 3.5 to 7.0. Possible foods include:

Home canned products having a low acidity (pH of 4.6 or greater)

Foods with low salt or low sugar content

Foods that are held at temperatures which allow the organism to grow (35°C, but as Low as 15°C.

Foods that are consumed without prior heating.

Unopened commercially processed cans are to be sent to the U.S. Food and Drug Administration with prior arrangements.

Transport – Refrigerated

Storage – Refrigerated – DO NOT FREEZE

Coxiella burnetii **"Q-fever"**

Serum –
Collect serum (red-top or serum separator tube, tiger-top tube) as soon as possible after onset of symptoms (acute phase) and with a follow-up specimen (convalescent phase) at 14-days for serological testing.

Transport – 2-8°C as soon as possible
Storage – 2-8°C

Blood –
Collect blood in EDTA (lavender) or sodium citrate (blue). Collect specimens prior to antimicrobial therapy.

Transport – 2-8°C
Storage – 2-8°C

Tissue, body fluids –
Collect aseptically and place tissue or body fluids in a sterile container. For small tissue samples, add several drops of sterile normal saline to keep the tissue moist.

Transport – 2-8°C (if transported within 24 hours)
Storage - -70°C

Nasopharyngeal swabs, bronchial/tracheal washings –
Collect aseptically.

Transport – 2-8°C
Storage – 2-8°C

Francisella tularensis **"Tularemia"**

Blood –
Collect per institution's procedure for routine blood cultures
A blue, purple, or green top tube may be requested for additional testing.

Transport – Directly to laboratory at room temperature
Storage – Room temperature

Biopsied tissue or scraping/aspirate of ulcer or lesion –
Collect aseptically and place tissue, scraping or aspirate in a sterile container. For small tissue samples, add several drops of sterile normal saline to keep the tissue moist.

Transport – Room temperature for immediate processing
Storage – If processing is delayed, store at 2-8°C.

Francisella tularensis **"Tularemia"**

Swabs –
Obtain a firm sample of the advancing margin of the lesion. If using a swab transport carrier, the swab should be reinserted in the transport package and the swab fabric moistened with the transport medium inside the packet.

Transport – 2-8°C; Room temperature is acceptable
Storage – 2-8°C

Sputum or aspirate –
Collect >1 ml of lower respiratory specimen in a sterile, leakproof container.

Transport -- <2 hours at room temperature
For transport >2 hours, transport at 2-8°C
Storage – 2-8°C

Serum –
For serological diagnosis, an acute phase specimen should be collected as soon as possible after onset of disease. A convalescent phase specimen should be collected 21 days after the acute specimen. Collect blood (a minimum of 5 ml) by venipuncture into a tube without anticoagulant. Allow blood to clot, separate serum into a separate tube.

Transport – 2-8°C as soon as possible
Storage – 2-8°C

Yersinia pestis **"Plague"**

Lower respiratory tract –
Collect at least 1 ml of a bronchial wash or transtracheal aspirate in a sterile, leakproof container. Sputum may be examined but it is not advised because of contamination by normal throat flora.

Transport – <2 hours, room temperature
If transport time >2 hours, transport at 2-8°C
Storage – Store at 2-8°C

Blood –
Collect per institution's procedure for routine blood cultures

Transport – Directly to laboratory at room temperature
Storage – Depends on instrument

Specimen Selection for Agents of Bioterrorism

Yersinia pestis "Plague"

Aspirate of involved tissue or biopsied specimen –
Collect liver, spleen, bone marrow and lung specimens into sterile leakproof containers. Aspirates may yield little material; therefore, a sterile saline flush may be needed to obtain an adequate amount of specimen. For small tissue samples, add 1-2 drops of sterile normal saline to keep the tissue moist.

Transport – Room Temperature for immediate processing
If processing will be delayed, transport at 2-8°C
Storage – 2-8°C

Vaccinia virus

Dried vesicular fluid on a slide –
Collect vesicular fluid by touching a clean glass light-microscope slide to the open lesion multiple times. Let slide air dry for 5 minutes. Place in a slide container for transport.

Transport – 2-8˚C
Storage – 2-8˚C

Vesicular swabs –
Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid.

Transport – Room Temperature
Storage – Room Temperature

Scabs –
Aseptically place scrapings/material into a sterile, leakproof, freezable container.

Transport - ~6 hours at 4˚C
Storage – (-)20˚C to (-)70˚C

Varicella zoster virus "Chickenpox"

Vesicular swabs –
Swabs of vesicular fluid should be collected by vigorously scrubbing the base of an unroofed lesion with a sterile swab. A polyester swab is preferred. Contamination with blood is not a concern for this test. Place swab in a snap cap tube or other suitable container. Break off the stick if necessary. Do not add transport fluid.

Transport – Room Temperature
Storage – Room Temperature

Varicella zoster virus "Chickenpox"

Scabs –
Aseptically place scrapings/material into a sterile, leakproof, freezable container.
Transport - ~6 hours at 4˚C
Storage – (-)20˚C to (-)70˚C

Variola virus "Smallpox"

A suspected case of smallpox should be reported immediately to the Utah Department of Health for review.

Biopsy specimens –
Aseptically place 2 to 4 portions of tissue into a sterile, leakproof, freezable container.
Transport - ~6 hours at 4˚C
Storage – (-)20˚C to (-)70˚C

Scabs –
Aseptically place scrapings/material into a sterile, leakproof, freezable container.
Transport - ~6 hours at 4˚C
Storage – (-)20˚C to (-)70˚C

Vesicular fluid –
Collect fluid from separate lesions onto separate sterile swabs. Be sure to include cellular material from the base of each respective vesicle.
Transport - ~6 hours at 4˚C
Storage – (-)20˚C to (-)70˚C

Viral Hemorrhagic Fever (VHF)

Specific handling conditions are currently under development at the CDC.

Serum –
Collect 10-12 cc of serum. Laboratory tests used at the CDC to diagnose VHF Include: antigen-capture ELISA, IgG ELISA, PCR and virus isolation.
Transport - ~2 hours at room temperature
Storage – (-)4˚C

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Bacillus anthracis* (Anthrax)
METHOD	N/A
AVAILABLE	All clients – Contact Utah State Public Health Lab prior to submitting test specimens (801)584-8449
PATIENT PREP	N/A
SPECIMEN	Environmental samples, organism isolate, cutaneous lesions, stool, rectal swab, blood cultures, whole blood, sputum, CSF, tissue, or nasal swab
COLLECT IN	Refer to *Bacillus anthracis* section on page 14.
PROCESSING	Refer to *Bacillus anthracis* section on page 14.
TRANSPORT	Refer to *Bacillus anthracis* section on page 14.
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	48 hours
RESULTS	Recovered or not recovered; Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801)584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Brucella* species (Brucellosis)
METHOD	N/A
AVAILABLE	All clients – Contact Utah State Public Health Lab prior to submitting test specimens (801)584-8449
PATIENT PREP	N/A
SPECIMEN	Organism isolate, environmental samples, blood, serum, spleen, liver or abscess
COLLECT IN	Refer to *Brucella* section on page 25.
PROCESSING	Refer to *Brucella* section on page 25
TRANSPORT	Refer to *Brucella* section on page 25
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	6 days
RESULTS	Recovered or not recovered; Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801)584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Burkholderia mallei* and *Burkholderia pseudomallei* (Glanders & Melioidiosis)
METHOD	N/A
AVAILABLE	All clients – Contact Utah State Public Health Lab prior to submitting test specimens (801)584-8449
PATIENT PREP	N/A
SPECIMEN	Organism isolate, blood, serum, urine, abscesses, tissue aspirates, body fluids, (throat, nasal, skin or sputum for intentional release exposures)
COLLECT IN	Refer to *Burkholderia* section on page 32.
PROCESSING	Refer to *Burkholderia* section on page 32.
TRANSPORT	Refer to *Burkholderia* section on page 32.
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	72 hours
RESULTS	Recovered or not recovered; Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801)584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Clostridium botulinum* (Botulism)
METHOD	Culture and toxin assay
AVAILABLE	Please contact UDOH Epidemiology prior to submitting test specimens (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Stool, enema fluid, gastric aspirate, vomitus, serum, tissue, wound, exudates, organism isolate, postmortem specimens, food and environmental samples
COLLECT IN	Refer to *Clostridium botulinum* section on page 42.
PROCESSING	Refer to *Clostridium botulinum* section on page 42.
TRANSPORT	Refer to *Clostridium botulinum* section on page 42.
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	48 to 96 hours
RESULTS	Recovered or not recovered
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801)584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Coxiella burnetii* (Q-fever)
METHOD	
AVAILABLE	All clients – Contact UDOH Lab prior to submitting test specimens (801) 584-8449
PATIENT PREP	N/A
SPECIMEN	Environmental samples, organism isolate, blood, serum, nasopharyngeal swab, bronchial/tracheal washing or lesion exudate
COLLECT IN	Refer to Coxiella burnetii section on page 46.
PROCESSING	Refer to Coxiella burnetii section on page 46.
TRANSPORT	Refer to Coxiella burnetii section on page 46.
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique identifier and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form
TEST COMPLETE	48 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax or email as established with provider
ADD. INFO	
CONTACT	Jana Coombs or Kim Christensen (801) 584-8449

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Francisella tularensis* (Tularemia)
METHOD	N/A
AVAILABLE	All clients – Contact Utah State Public Health Lab prior to submitting test specimens (801)584-8449
PATIENT PREP	N/A
SPECIMEN	Organism isolate, environmental samples, blood cultures, biopsied tissue, ulcer or lesion scraping or aspirate, lesion swabs, sputum, bronchial/tracheal wash, serum for serological diagnosis
COLLECT IN	Refer to *Francisella tularensis* section on page 49.
PROCESSING	Refer to *Francisella tularensis* section on page 49
TRANSPORT	Refer to *Francisella tularensis* section on page 49
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	48 hours
RESULTS	Recovered or not recovered; Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	Ricin toxin
METHOD	N/A
AVAILABLE	Ordered by Epidemiology, Local Health, Local Law, or FBI
PATIENT PREP	N/A
SPECIMEN	Environmental samples
COLLECT IN	Original container or sterile container
PROCESSING	Use universal precautions – all manipulations under a Biosafety Cabinet
TRANSPORT	Refer to Safe Handling, Packaging and Shipping Guidelines
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Identification, sample description, date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Staphylococcus* enterotoxin B (SEB)
METHOD	N/A
AVAILABLE	Ordered by Epidemiology, Local Health, Local Law, or FBI
PATIENT PREP	N/A
SPECIMEN	Environmental samples
COLLECT IN	Original container or sterile container
PROCESSING	Use universal precautions – all manipulations under a Biosafety Cabinet
TRANSPORT	Refer to Safe Handling, Packaging and Shipping Guidelines
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Identification, sample description, date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

TEST	Vaccinia virus
METHOD	N/A
AVAILABLE	All Clients – Contact UDOH Epidemiology prior to submitting test specimens (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Microscope slide touch preps, scabs, dried vesicular fluid, vesicular swabs
COLLECT IN	Refer to Variola virus section on page 56
PROCESSING	Refer to Variola virus section on page 56
TRANSPORT	Refer to Variola virus section on page 56
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	Refer to the Smallpox Specimen Information link on the Microbiology website (health.utah.gov/els/microbiology)
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

TEST	Varicella zoster virus (Chickenpox)
METHOD	N/A
AVAILABLE	All Clients – Contact UDOH Epidemiology prior to submitting test specimens (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Vesicular swab (cotton or Dacron polyester), scabs from crusted lesions
COLLECT IN	Refer to Variola virus section on page 56
PROCESSING	Refer to Variola virus section on page 56
TRANSPORT	Refer to Variola virus section on page 56
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	Refer to the Smallpox Specimen Information link on the Microbiology website (health.utah.gov/els/microbiology)
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

TEST	Variola virus (Smallpox)
METHOD	N/A
AVAILABLE	All Clients – Contact UDOH Epidemiology prior to submitting test specimens (801)538-6191
PATIENT PREP	N/A
SPECIMEN	Microscope slide touch preps, scabs, dried vesicular fluid, vesicular swabs, vesicular tissue
COLLECT IN	Refer to Variola virus section on page 56
PROCESSING	Refer to Variola virus section on page 56
TRANSPORT	Refer to Variola virus section on page 56
TIME CRITICAL	Should be received at our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	24 hours
RESULTS	Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	Refer to the Smallpox Specimen Information link on the Microbiology website (health.utah.gov/els/microbiology)
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen

STATE OF UTAH PUBLIC HEALTH LABORATORY
MICROBIOLOGY CLIENT SERVICES MANUAL
TESTS – Bioterrorism Section

TEST	*Yersinia pestis* (Plague)
METHOD	N/A
AVAILABLE	All Clients – Contact Utah State Public Health Lab prior to submitting test specimens (801) 584-8449
PATIENT PREP	N/A
SPECIMEN	Isolate of organism, environmental samples, bronchia wash, tracheal Aspirate, sputum, nasopharyngeal swabs, lymph node aspirates, serum, lesion exudates, tissue smears, blood
COLLECT IN	Refer to *Yersinia pestis* section on page 59
PROCESSING	Refer to *Yersinia pestis* section on page 59
TRANSPORT	Refer to *Yersinia pestis* section on page 59
TIME CRITICAL	Should be received in our laboratory as soon as possible
LABEL	Patient's full name or unique ID number, and date of collection or subculture
REQUISITION	Bioterrorism Test Request Form (see form in Appendix B)
TEST COMPLETE	3 days
RESULTS	Recovered or not recovered; Detected or not detected
REPORTED	Phone, fax, or email, as established with provider
NOTE	N/A
CONTACT	(801) 584-8449: Jana Coombs or Kim Christensen